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(54) Title: RECOMBINANT AND CHIMERIC ANTIBODIES TO c-erbB-2

(57) Abstract

This invention relates to methods for producing recombinant and chimeric antibody peptides that recognize the c-erbB-2 oncogene, and to vectors, host cells and DNA sequences for producing such antibody peptides.

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RECOMBINANT AND CHIMERIC ANTIBODIES TO c-erbB-2

Field of the Invention

This invention relates to methods for producing recombinant and chimeric antibody peptides that recognize the c-erbB-2 oncogene protein, and to vectors, host cells and DNA sequences for producing such antibody peptides.

Background of the Invention

The mechanism for malignancy of mammalian cells has been and continues to be the subject of intense investigation. A number of oncogenes, some of which encode proteins that regulate cell growth and differentiation, have been shown to play an important role in causing cancer. Many of the proteins encoded by oncogenes function abnormally in malignant cells or are not regulated by normal control processes. This seems to play a part in the transformation of normal cells into cancer cells.

The c-erbB-2 protein (also referred to as c-erbB-2 protein receptor and sometimes designated HER-2 or neu) is a 185 kilodalton (Kd) glycoprotein having tyrosine kinase activity and is related to, but distinct from, the epidermal growth factor receptor (EGFR). Over-expression of c-erbB-2 has been linked to poor clinical outcome and shortened disease-free survival of mammary and ovarian cancer patients. Slamon

et al., *Science*, 235:177 (1987); Berchuck, A., et al., *Cancer Res.*, 50:4087 (1990).

Antibodies are molecules that recognize and bind to a specific cognate antigen. Numerous applications of hybridoma-produced monoclonal antibodies for use in clinical diagnoses, treatment, and basic scientific research have been described. Clinical treatments of cancer, viral and microbial infections, B cell immunodeficiencies, and other diseases and disorders of the immune system using monoclonal antibodies appear promising. In particular, TAb 250, a mouse monoclonal antibody against the c-erbB-2 protein has been shown to be cytostatic for tumor cell growth. See Hancock et al., *Can. Res.*, 51:4575 (1991).

In addition, promising monoclonal antibodies have been shown to interact synergistically with anti-neoplastic drugs. For example, an antibody to c-erbB-2 protein has been shown to synergistically enhance the anti-tumor activity of the drug cisplatin. Hancock et al., *J. Cell. Biochem. Supp.*, 148:342 (1990) and Hancock et al., *Can. Res., supra.*

Mouse monoclonal antibody production is well known in the art, and mouse monoclonal antibodies can be generated against a wide array of antigens. Clinical results, however, of some immunotherapies have been disappointing. One possible source of the problems associated with cancer immunotherapy is the patient's own immune response to the therapeutic antibody. When used for clinical treatment in humans, the mouse antibody is recognized as antigen and the human immune system mounts a response. The human response to the mouse antibodies poses two potential problems. First, the anti-mouse immunoglobulin response can cause harmful symptoms. Second, the response can prematurely clear the therapeutic antibody from the patient's system, reducing its effectiveness and requiring higher doses. Although treatment with human monoclonal antibodies has been

investigated, human hybridoma cell lines tend to be unstable and produce low amounts of immunoglobulin.

One approach to circumvent the immunogenicity of non-human antibodies in humans is to use recombinant DNA techniques to incorporate the segments of a non-human antibody that determine the antibody's specificity with other segments of a human antibody. Much of the immunogenicity resides in the constant domains of the non-human antibody. For example, mouse variable region exons specific for tumor antigens have been fused with human κ or γ constant regions. See, for example, Sahagan et al., *J. Immunol.*, 1066 (1986); Liu et al., *Proc. Natl. Acad. Sci. USA*, 84:3439 (1987); and Steplewski et al., *Proc. Natl. Acad. Sci. USA*, 85:4852 (1988). A variation of this approach uses DNA sequences that code only complementarity-determining regions, also known as CDRs, from a mouse or other foreign antibody specific for the antigen of interest.

One advantage of such chimeric forms is that the variable regions can conveniently be derived from presently known sources using readily available hybridomas or B cells from non-human host organisms. These variable regions can be combined with constant regions derived from, for example, human cell preparations. Use of the human constant region is less likely to elicit an immune response when the antibodies are injected into a human subject. In addition, the human constant regions may interact more effectively with the human effector cells of the immune system. Thus, it would be beneficial to develop such chimeric antibodies in cancer therapy.

Brief Description of the Drawings

Various other objects, features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood when considered in conjunction

with the accompanying drawings, in which like reference characters designate the same or similar parts throughout the several views, and wherein:

Figure 1 illustrates the cloning of a PCR-amplified mouse V_H fragment from the DNA of hybridoma cells producing an antibody to c-erbB-2. Plasmid pUC19 was cut with Eco RI and then treated with calf intestine alkaline phosphatase. The purified V_H fragment was then ligated into the Eco RI site to make pUC19V_H;

Figure 2 illustrates the cloning of a PCR-amplified mouse V_K (V_L) region from the DNA of the hybridoma producing the same antibody to c-erbB-2 as in Figure 1. Plasmid pIBI21 was cut with Hind III and treated with phosphatase. The purified V_K fragment was cloned into the Hind III site to make the plasmid pIBI21V_K;

Figure 3 illustrates the cloning of a PCR-amplified mouse κ enhancer. Plasmid pUC19 was cut with Eco RI and treated with calf intestine alkaline phosphatase. The enhancer was ligated into the Eco RI site to make p19enhancer;

Figure 4 illustrates the cloning of a PCR-amplified human $\gamma 1$ constant region from the DNA of ARH-77 cells. Plasmid pBR322 was cut with Bam HI and Sal I and then treated with calf intestine alkaline phosphatase. The $C\gamma 1$ fragment was then ligated into the Bam HI and Sal I sites to make plasmid p322$\gamma 1$;

Figure 5 illustrates the cloning of a PCR-amplified human C_K constant region from the DNA of ARH-77 cells. Plasmid pUC19 was cut with Bam HI and Eco RI and then treated with calf intestine alkaline phosphatase. The C_K fragment was then ligated into the Bam HI/Eco RI site to make plasmid p19κ;

Figure 6 illustrates the first step in construction of a chimeric heavy chain expression vector: cloning the mouse V_H region into pBR322. Plasmid pUC19V_H was digested with Eco RI

and Sal I and purified V_H fragment was subcloned into pBR322 at the Bam HI and Sal I sites with the use of a Bam HI and Sal I oligonucleotide adapter, to create p322V_H;

Figure 7 illustrates the second step in construction of a chimeric heavy chain expression vector: subcloning the mouse V_H and Human Cγ1 fragment into plasmid pSV2neo. The mouse V_H and the human Cγ1 fragments were excised from p322V_H and p322γ1, respectively, by cutting with Bam HI and Sal I. Plasmid pSV2neo was cut with Bam HI and treated with calf intestine alkaline phosphatase. The V_H and Cγ1 fragments were ligated into the pSV2neo vector at the Bam HI site to make pSVNH-L;

Figure 8 illustrates the first step in construction of a chimeric light chain expression vector: subcloning mouse Vκ fragment into pBR322. Plasmid pBR322 was cut with Hind III and treated with calf intestine alkaline phosphatase. The V_K fragment was excised from pIBI21V_K by cutting with Hind III. The V_K fragment was ligated into pBR322 at the Hind III site to make p322V_K;

Figure 9 illustrates construction of p322ΔRH. Plasmid pBR322 was cut with Eco RI and Hind III and then treated with Klenow fragment of DNA polymerase. The plasmid ends were ligated to make p322ΔRH;

Figure 10 illustrates the construction of a plasmid containing a mouse V_K and human C_K. The mouse V_K and the human C_K region were excised from p322V_K and p19κ, respectively, by cutting with both Bam HI and Eco RI. Plasmid p322ΔRH was cut with Bam HI and treated with calf intestine alkaline phosphatase. The V_K and the C_K fragments were ligated into the Bam HI site to make plasmid pΔRH-light;

Figure 11 illustrates the construction of an intermediate plasmid containing a mouse V_K and human C_K. Plasmid pΔRH-light

was cut with Eco RI and Xba I. An Eco RI-Xba I adapter was ligated into the site to make pΔ-light;

Figure 12 illustrates the construction of a plasmid containing DNA encoding a mouse V_κ, a mouse κ enhancer, and human C_κ region. Plasmid pΔ-light was cut with Eco RI and treated with calf intestine alkaline phosphatase. The mouse κ enhancer was excised from p19enhancer by cutting with Eco RI. The enhancer was ligated into the Eco RI site of the cut pΔ-light to make the plasmid pΔL-enhancer containing a chimeric κ chain;

Figure 13 illustrates the final step in constructing a chimeric light chain expression vector. Plasmid pRSV-gpt was cut with Bam HI and treated with calf intestine alkaline phosphatase. The chimeric κ chain DNA was excised from pΔL-enhancer by cutting with Bam HI. The Bam HI fragment containing the chimeric light chain was ligated into the Bam HI site of pRSV-gpt to make pRGL-L, a chimeric light chain expression vector;

Figure 14 illustrates the ability of mouse/human chimeric antibodies (clones E8 and A7) to displace the binding of a murine monoclonal antibody, TAb 250, to SKBR-3 cells bearing the c-erbB-2 protein;

Figure 15 illustrates that BACh 250 mouse/human chimeric antibodies suppressed proliferation of SKOV3 cells bearing the c-erbB-2 protein in a manner similar to TAb 250, or the Fab or $F(Ab')_2$ fragments of TAb 250; and

Figures 16A and B indicate that BACh 250 fixes rabbit complement more effectively than TAb 250 by measuring chromium release from SKBR-3 cells in the presence of the antibodies and rabbit complement.

Summary of the Invention

Chimeric or recombinant antibody peptides specific for c-rbB-2 can be used as important therapeutic or diagnostic agents for treating mammary and ovary cancers, or other tumors expressing significant levels of c-erbB-2. For example, these chimeric antibodies and peptides could be used to trigger the patient's endogenous immune effector response; be covalently linked to a toxin or radionuclide and used as an immunotoxin; or be administered in combination with a cytotoxic or cytostatic agent. A murine monoclonal antibody to c-erbB-2 protein, TAb 250, does not mediate human immune effector functions including complement dependent cellular cytotoxicity and antibody dependent cellular cytotoxicity. A mouse/human antibody improves such functions while maintaining the chemotherapeutic effects of the antibody.

The invention includes recombinant DNA sequences encoding at least one CDR region derived from an antibody specific for c-erbB-2 protein. The CDR region may be derived from a heavy chain or light chain variable region of the antibody. In a preferred embodiment the CDR regions will be derived from an antibody that activates the signalling pathway of the c-erbB-2 protein. Such activation is indicated, for example, when the antibody induces an increase in phosphorylation of the c-erbB-2 protein, causes down modulation of the protein, or induces phosphorylation of substrates such as phospholipase C (PLC-γ1) when placed in contact with cells expressing the c-erbB-2 protein.

Further included are recombinant DNA sequences that encode an antibody heavy chain variable region or light chain variable region specific for c-erbB-2 protein. These sequences may be those which encode variable regions that, when combined with a complementary chain (heavy/light)

(including those set forth in Sequence ID Nos. 1 or 2), specifically bind c-erbB-2.

The invention also includes recombinant DNA sequences that encode an antibody light chain variable region or heavy chain variable region which, when incorporated into immunoglobulin conformation, competes with an antibody produced by a hybridoma cell line bearing A.T.C.C. Accession No. HB10646 (TAb 250) for the binding to c-erbB-2. Preferred sequences include those that comprise DNA encoding an antibody heavy chain variable region or light chain variable region to c-rbB-2 protein having an amino acid sequence consisting essentially of that sequence set forth in Sequence ID No. 3 or 4, respectively.

Recombinant vectors, host cells, and chimeric antibody peptides, e.g., humanized antibody peptides are also encompassed in the invention. For example, included are recombinant DNA sequences that comprise DNA encoding a chimeric c-rbB-2 specific heavy chain peptide, the variable region derived from a first genetic source and a constant region derived from a second and different genetic source. Also included are sequences that comprise DNA encoding a chimeric c-erbB-2 specific light chain peptide, the variable region derived from a first genetic source and a constant region derived from a second and different genetic source.

Detailed Description

The present invention encompasses recombinant DNA sequences, recombinant vectors, host cells, and immunoglobulin peptides (including CDRs) encoded by recombinant sequences. Particularly of interest are sequences encoding chimeric immunoglobulin peptides that specifically recognize the c-erbB-2 antigen.

The c-erbB-2 protein (also referred to here simply as c-rbB-2) is a 185 Kd (Kilodalton) membrane glycoprotein having tyrosine kinase activity and is related to, but distinct from, the epidermal growth factor receptor (EGFR). Like the EGFR protein, the c-erbB-2 protein has an extracellular domain that includes two cysteine-rich repeat clusters, a transmembrane domain and an intracellular kinase domain. In addition, the amino acid sequence of the c-erbB-2 protein as well as the nucleotide sequence has been described by Coussens et al., *Science*, 230:1132 (1985), incorporated by reference herein. The c-erbB-2 protein is encoded by the c-erbB-2 oncogene described in 1985 by three different research groups: Semba et al., *Proc. Natl. Acad. Sci. USA*, 82:6497 (designating the gene as c-erbB-2); Coussens et al., *supra*, (designating the gene as HER-2); and King et al., *Science*, 229:1132 (designating the gene as v-erbB related). Thus, the c-erbB-2 gene sequence and its corresponding protein sequence are well-known and described in the art. Detection of the c-erbB-2 protein may be accomplished by well-known immunoassays employing antibodies specific to the c-erbB-2 protein, such as those described here. Such antibodies are commercially available, for example, from Chemicon International, Inc., Temecula, CA or may be prepared by standard immunological procedures. See, for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Publications, N.Y. (1988), incorporated by reference herein. It is intended herein that the c-erbB-2 protein definition will also include those proteins developed from other host systems, e.g., proteins that are immunologically related to the human c-erbB-2 protein. For example, a related rat gene (designated *neu*) has been reported in Schecter et al., *Science*, 229:976 (1985).

One objective of this invention is to provide recombinant immunoglobulin peptides which bind to c-erbB-2. Immunoglobu-

lins or antibodies are typically composed of four covalently bound peptide chains. For example, an IgG antibody has two light chains and two heavy chains. Each light chain is covalently bound to a heavy chain. In turn each heavy chain is covalently linked to the other to form a "Y" configuration, also known as an immunoglobulin conformation. Fragments of these molecules, or even heavy or light chains alone, may bind antigen. Antibodies, fragments of antibodies, and individual chains are also referred to herein as immunoglobulins.

A normal antibody heavy or light chain has an N-terminal (NH_2) variable (V) region, and a C-terminal (-COOH) constant (C) region. The heavy chain variable region is referred to as V_H (including, for example, V_γ), and the light chain variable region is referred to as V_L (including V_κ or V_λ). The variable region is the part of the molecule that binds to the antibody's cognate antigen, while the Fc region (the second and third domains of the C region) determines the antibody's effector function (e.g., complement fixation, opsonization). Full-length immunoglobulin or antibody "light chains" (generally about 25 Kd, about 214 amino acids) are encoded by a variable region gene at the N-terminus (generally about 110 amino acids) and a κ (kappa) or λ (lambda) constant region gene at the COOH-terminus. Full-length immunoglobulin or antibody "heavy chains" (generally about 50 Kd, about 446 amino acids), are similarly encoded by a variable region gene (generally encoding about 116 amino acids) and one of the constant region genes, e.g. gamma (encoding about 330 amino acids). Typically, the "V_L" will include the portion of the light chain encoded by the V_L and/or J_L (J or joining region) gene segments, and the "V_H" will include the portion of the heavy chain encoded by the V_H, and/or D_H (D or diversity region) and J_H gene segments. See generally, Roitt et al., *Immunology*, Chapter 6, (2d ed. 1989) and Paul, *Fundamental*

Immunology; Raven Press (2d ed. 1989), both incorporated by reference herein.

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called complementarity-determining regions or CDRs. The extent of the framework region and CDRs have been defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1987); which is incorporated herein by reference). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three dimensional space. The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus.

The two types of light chains, κ and λ, are referred to as isotypes. Isotypic determinants typically reside in the constant region of the light chain, also referred to as the C_L in general, and C_κ or C_λ in particular. Likewise, the constant region of the heavy chain molecule, also known as C_H, determines the isotype of the antibody. Antibodies are referred to as IgM, IgD, IgG, IgA, and IgE depending on the heavy chain isotype. The isotypes are encoded in the mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ϵ) segments of the heavy chain constant region, respectively. In addition, there are a number of γ subtypes.

The heavy chain isotypes determine different effector functions of the antibody, such as opsonization or complement fixation. In addition, the heavy chain isotype determines the secreted form of the antibody. Secreted IgG, IgD, and IgE

isotypes are typically found in single unit or monomeric form. Secreted IgM isotype is found in pentameric form; secreted IgA can be found in both monomeric and dimeric form.

Mouse monoclonal antibodies have been made against the extracellular portion of c-erbB-2. An example of such an antibody is TAb 250, which is deposited with the American Type Culture Collection, Rockville, Maryland (ATCC) bearing Accession No. HB10646.

The DNA sequences of this invention comprise DNA subsequences encoding amino acid sequences of the antibody heavy or light chains, or fragments thereof, which determine binding specificity for the oncogene c-erbB-2 protein such as those derived from TAb 250. These sequences may be ligated, for example, into human constant region expression vectors, and inserted into a host cell. The host cell can then express a recombinant chimeric or hybrid antibody that is specific for binding to a c-erbB-2 protein or polypeptide.

"Immunoglobulin" or "antibody peptide(s)" refers to an entire immunoglobulin or antibody or any functional fragment of an immunoglobulin molecule. Examples of such peptides include complete antibody molecules, antibody fragments, such as Fab, $F(ab')_2$, CDRs, V_L, V_H, and any other portion of an antibody. As described above, an IgG antibody molecule is composed of two light chains linked by disulfide bonds to two heavy chains. The two heavy chains are, in turn, linked to one another by disulfide bonds in an area known as the hinge region of the antibody. A single IgG molecule typically has a molecular weight of approximately 150-160 kD and containing two antigen binding sites.

An $F(ab')_2$ fragment lacks the C-terminal portion of the heavy chain constant region, and has a molecular weight of approximately 110 kD. It retains the two antigen binding sites and the interchain disulfide bonds in the hinge region,

but it does not have the effector functions of an intact IgG molecule. An $F(ab')_2$ fragment may be obtained from an IgG molecule by proteolytic digestion with pepsin at pH 3.0-3.5 using standard methods such as those described in Harlow and Lane, *supra*.

An "Fab" fragment comprises a light chain and the N-terminus portion of the heavy chain to which it is linked by disulfide bonds. It has a molecular weight of approximately 50 kD and contains a single antigen binding site. Fab fragments may be obtained from $F(ab')_2$ fragments by limited reduction, or from whole antibody by digestion with papain in the presence of reducing agents. (See, Harlow and Lane, supra.) In certain cases, the concentration of reducing agent necessary to maintain the activity of papain in the presence of atmospheric oxygen is sufficient to fully reduce the interchain disulfide bonds to the antibody. This can result in loss of antigen recognition. To circumvent this problem, papain may be activated and then exchanged into buffer containing a concentration of reducing agent compatible with maintaining antigen binding activity. The antibody digestion is carried out under an inert atmosphere to prevent deactivation of the papain.

The following protocol is an example of this process:

A) Activation of papain: Papain, supplied as 10 mg/ml NH_4SO_4 suspension, is dissolved in 10 mM EDTA, 20 mM cysteine, pH=8.0, to a final concentration of 2 mg/ml. The solution is degassed and allowed to incubate 2 hours at room temperature under nitrogen.

B) The activated papain is exchanged into 20 mM $NaPO_4$, pH=7.0, 150 mM NaCl, 10 mM EDTA, 30 μM DTT.

C) Digestion of antibody: 1 mg of activated papain is added for every 100 mg of antibody, and the solution is dialyzed against a large excess of 20 mM $NaPO_4$, pH=7.0, 150 mM

NaCl, 10 mM EDTA, 30 μM DTT, with continuous helium sparging. Dialysis is necessary to maintain a molar excess of reducing agent during the course of the digestion.

D) After 2-4 hours at room temperature the digestion is terminated by addition of iodoacetamide.

E) Fab fragments are separated from undigested or partially digested antibody using standard chromatography methods.

"Fab", or any other antibody fragment, has similar classifications according to the definition of the present invention as does the general term "antibodies" or "immunoglobulins". Thus, "mammalian" Fab protein, "chimeric Fab", and the like are defined analogously to the corresponding definitions set forth in the subsequent paragraphs.

"Chimeric antibodies" or "chimeric peptides" refer to those antibodies or antibody peptides wherein one portion of the peptide has an amino acid sequence that is derived from, or is homologous to a corresponding sequence in an antibody or peptide derived from a first gene source, while the remaining segment of the chain(s) is homologous to corresponding sequences of another gene source. For example, a chimeric antibody peptide may comprise an antibody heavy chain with a murine variable region and a human constant region. The two gene sources will typically involve two species, but will occasionally involve one species.

Chimeric antibodies or peptides are typically produced using recombinant molecular and/or cellular techniques. Typically, chimeric antibodies have variable regions of both light and heavy chains that mimic the variable regions of antibodies derived from one mammalian species, while the constant portions are homologous to the sequences in antibodies derived from a second, different mammalian species.

The definition of chimeric antibody, however, is not limited to this example. A chimeric antibody is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, differing antigen responses, or differing species of origin, and whether or not the fusion point is at the variable/constant boundary. For example, chimeric antibodies can include antibodies where the framework and complementarity- determining regions are from different sources. For example, non-human CDRs are integrated into human framework regions linked to a human constant region to make "humanized antibodies." See, for example, PCT Application Publication No. WO 87/02671, U.S. Patent No. 4,816,567, EP Patent Application 0173494, Jones, et al., *Nature*, 321:522-525 (1986) and Verhoeyen, et al., *Science*, 239:1534-1536 (1988), all of which are incorporated by reference herein.

A "human-like framework region" is a framework region for each antibody chain, and it usually comprises at least about 70 or more amino acid residues, typically 75 to 85 or more residues. The amino acid residues of the human-like framework region are at least about 80%, preferably about 80-85%, and most preferably more than 85% homologous with those in a human immunoglobulin.

The term "humanized" or "human-like immunoglobulin" refers to an immunoglobulin comprising a human-like framework region and a constant region that is substantially homologous to a human immunoglobulin constant region, e.g., having at least about 80% or more, preferably about 85-90% or more and most preferably about 95% or more homology. Hence, most parts of a human-like immunoglobulin, except possibly the CDRs, are substantially homologous to corresponding parts of one or more native human immunoglobulin sequences.

"Hybrid antibody" refers to an antibody wherein each chain is separately homologous with reference to a mammalian antibody chain, but the combination represents a novel assembly so that two different antigens are recognized by the antibody. In hybrid antibodies, one heavy and light chain pair is homologous to that found in an antibody raised against one epitope, while the other heavy and light chain pair is homologous to a pair found in an antibody raised against another epitope. This results in the property of multifunctional valency, i.e., ability to bind at least two different epitopes simultaneously. Such hybrids may, of course, also be formed using chimeric chains.

The present invention, *inter alia*, encompasses a chimeric antibody, including a hybrid antibody or a humanized or human-like antibody. It also encompasses a recombinant DNA sequence encoding segments of said antibody or any peptide specific for c-erbB-2 protein. In a preferred embodiment, the variable sequence originates from and is substantially identical to a sequence of the murine TAb 250 antibody as described in Sequence ID Nos. 1 or 2, and is combined with human $\gamma 1$ and κ constant regions.

In the case of the sequences described herein, it should be understood that variants of these sequences are also included, such as substitution, addition, and/or deletion mutations, or any other sequence possessing substantially similar binding activity to the sequences from which they are derived or otherwise similar to.

For this invention, an antibody or other peptide is specific for a c-erbB-2 protein if the antibody or peptide binds or is capable of binding c-erbB-2 protein as measured or determined by standard antibody-antigen or ligand-receptor assays, for example, competitive assays, saturation assays, or standard immunoassays such as ELISA or RIA. This definition

of specificity applies to single heavy and/or light chains, CDRs, fusion proteins or fragments of heavy and/or light chains, that are also specific for c-erbB-2 protein if they bind c-erbB-2 protein alone or if, when properly incorporated into immunoglobulin conformation with complementary variable regions and constant regions as appropriate, are then capable of binding c-erbB-2 protein.

In competition assays the ability of an antibody or peptide fragment to bind an antigen is determined by detecting the ability of the peptide to compete with the binding of a compound known to bind the antigen. Numerous types of competitive assays are known and are discussed herein. Alternatively, assays that measure binding of a test compound in the absence of an inhibitor may also be used. For instance, the ability of a molecule or other compound to bind the c-erbB-2 protein can be detected by labelling the molecule of interest directly or it may be unlabelled and detected indirectly using various sandwich assay formats. Numerous types of binding assays such as competitive binding assays are known (see, e.g., U.S. Patent Nos. 3,376,110, 4,016,043, and Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Publications, N.Y. (1988), which are incorporated herein by reference). Assays for measuring binding of a test compound to one component alone rather than using a competition assay are also available. For instance, immunoglobulins can be used to identify the presence of the c-erbB-2 protein. Standard procedures for monoclonal antibody assays, such as ELISA, may be used (see, Harlow and Lane, *supra*). For a review of various signal producing systems which may be used, see, U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Further, the specificity of the peptides to c-erbB-2 can be determined by their affinity for the antigen. Such specificity exists if the dissociation constant (K_D = 1/K, where K

is the affinity constant) f the peptides is < 1μM, preferably < 100 nM, and most preferably < 1 nM. Antibody molecules will typically have a K_D in the lower ranges. K_D = [R-L]/[R][L] where [R], [L], and [R-L] are the concentrations at equilibrium of the receptor or c-erbB-2 (R), ligand or peptide (L) and receptor-ligand complex (R-L), respectively. Typically, the binding interactions between ligand or peptide and receptor or antigen include reversible noncovalent associations such as electrostatic attraction, Van der Waals forces and hydrogen bonds.

Other assay formats may involve the detection of the presence or absence of various physiological or chemical changes that result from the interaction, such as down modulation, internalization or an increase in phosphorylation as described in United States Patent Application No. 07/644,361 filed January 18, 1991, incorporated by reference herein. See also, *Receptor-Effector Coupling - A Practical Approach*, ed. Hulme, IRL Press, Oxford (1990).

A preferred peptide specific for c-erbB-2 protein induces an increase in the phosphorylation of the c-erbB-2 protein when placed in contact with tumor cells expressing the c-erbB-2 protein. A molecule that "induces an increase in the phosphorylation of c-erbB-2 protein" is one that causes a detectable increase in the incorporation of phosphate into the protein over that which occurs in the absence of the molecule. Typically this detectable increase will be a two-fold or greater increase in phosphorylation, preferably greater than a three-fold increase over controls. Phosphorylation may be measured by those methods known in the art for detecting phosphorylation of receptors. See, for example Cooper et al., *Methods in Enzymology*, 99:387-402 (1983); Antoniades and Pantazis, *Methods in Enzymology*, 147:36-40 (1987); and Lesniak

et al., *Methods in Enzymology*, 150:717-723 (1987), which are all incorporated by reference herein.

Typically, phosphorylation can be measured by *in vivo* phosphorylation of intact cells (Lesniak, *supra*) or by an *in vitro* autophosphorylation reaction (Antonaides, *supra*). For measuring *in vivo* phosphorylation, for example, assays may be conducted where cells bearing the c-erbB-2 protein are placed into contact with radioactive labelled phosphate. To detect phosphorylation of the c-erbB-2 protein receptor in the *in vivo* assay, it is advantageous to incubate the test cells for about 12 to about 18 hours, with the labeled phosphate. The cells are divided into two or more batches, where some are exposed to the molecule expected to increase the phosphorylation of the receptor and some are separated out as controls. The aliquots are subsequently immunoprecipitated, the receptor is recognized, for example, by SDS polyacrylamide gel or autoradiography methods, and an increase in phosphorylation is considered statistically significant when when there is a two-fold or greater increase in the background of the aliquot exposed to the test molecule over the control aliquots.

To measure *in vitro* autophosphorylation, for example, cells or cell extracts may be incubated in the presence or absence of the peptide specific for c-erbB-2. Following immunoprecipitation with an anti-c-erbB-2 antibody, the immune complex may be incubated with γ^{32}P-ATP and analyzed by SDS-PAGE autoradiography.

Another preferred peptide specific for c-erbB-2 protein is one that causes down modulation of the c-erbB-2 protein. "Down modulation of the c-erbB-2 protein" is determined by a detectable decrease in the presence on the tumor cells of the c-erbB-2 receptor. Such down modulation is detected by a decrease in the ability of antibodies or other specific binding moieties to bind to or recognize the c-erbB-2 receptor

protein on the tumor cells. For example, down modulation can be determined by incubating tumor cells bearing the c-erbB-2 protein receptor with the peptide of interest, washing the cells, then contacting the cells with labeled (preferably radiolabelled) antibodies specific for the c-erbB-2 protein. The extent of binding of the labelled anti-c-erbB-2 antibodies to the cells exposed to the peptide specific for c-erbB-2 protein is compared to the extent of binding of the antibodies to control cells (i.e., not exposed to the c-erbB-2 specific peptide). Preferably for these assays, the cells are directly subjected to the labeled anti-c-erbB-2 antibodies after washing.

The down modulation observed is typically dose dependent, i.e., the extent of down modulation increases with the amount of peptide specific for c-erbB-2 protein exposed to the c-erbB-2 protein. Preferably, a peptide that causes a decrease in 90% or greater of binding of the treated cells versus control cells to anti-c-erbB-2 antibodies is desirable.

Another preferred peptide specific for c-erbB-2 protein is one that binds tumor cells expressing c-erbB-2 protein and is internalized when placed in contact with such tumor cells. "Internalization" occurs when the receptor becomes sequestered in the cytoplasm of the cells. Once internalized, the receptor may be degraded in the cell lysosomes or may be recycled to the cell surface. A method for determining internalization of a ligand-receptor complex is also described in Haigler et al., *J. Biol. Chem.*, 255:1239-1241 (1980), incorporated by reference herein.

This invention further includes recombinant DNA vectors comprising a gene expression control DNA sequence operably linked to the antibody peptide coding sequence. Examples of such a control sequence include a naturally-associated or heterologous promoter region. Preferably, the expression

control s quence will be a eukaryotic promoter system in a vector capable of transforming or transfecting a eukaryotic host cell. A control sequence for a prokaryotic host, however, can also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequence, and, as desired, the collection and purification of the light chain, heavy chain, light/heavy chain dimer or intact antibodies, binding fragments or other immunoglobulin form may follow. See generally for construction of expression vectors, Kriegler, *Gene Transfer and Expression*, M.H. Freeman, N.Y., N.Y. (1990), which is incorporated by reference.

The vectors of this invention also include recombinant DNA sequences encoding an antibody, or antibody peptide, along with a relevant transcriptional element, such as an enhancer and/or promoter. These transcriptional elements will be operably linked to the encoding gene to ensure their expression in the host cell system.

Other aspects of this invention include, for example, recombinant DNA sequences comprising one or more of the CDRs of antibody peptides that compete with TAb 250 for binding to c-erbB-2. They may be interspersed among framework regions, including those derived from a different species.

This invention further includes suitable host cell lines. For example, the sequences encoding the anti-c-erbB-2 antibody peptides are placed into expression vectors for transfection or transduction into bacteria, yeast, amphibian oocytes, insect cells or mammalian host cell lines, such as myeloma cells, Cos, CHO or L cells. See generally, Kriegler, *supra*, Sambrook et al, *Molecular Cloning: A Laboratory Manual* (2d ed. 1989), European Patent Application Publication No. 0125023, published 11/14/84; Kameyama et al., *FEBS.*, 244:301 (1989);

Better et al., *Science*, 240:1041 (1988), all of which are incorporated by reference.

Using standard methods that are well known in the art, the variable regions and CDRs may be derived from a hybridoma that produces a monoclonal antibody that is specific for c-erbB-2. The nucleic acid sequences of the present invention capable of ultimately expressing the desired chimeric antibodies can be formed from a variety of different nucleotide sequences (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently a common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and Reichmann et al., *Nature*, 332:323-327 (1988), both of which are incorporated herein by reference).

In one preferred embodiment, the sequences encoding the V_L and V_H regions are cloned from a hybridoma's genomic DNA, or cDNA produced by reverse transcription of the hybridoma's RNA. See Sambrook et al., *supra*. Cloning can be accomplished using traditional techniques, including the use of PCR primers that hybridize to the sequences flanking or overlapping with the variable regions or CDRs to amplify sequences of interest using cDNA or genomic DNA, as described below. See, Orlandi et al., *Proc. Natl. Acad. Sci. USA*, 86:3833 (1989), which is incorporated by reference. Exemplary primers for a variable heavy chain sequence are set out in Sequence ID Nos. 17 and 18. Exemplary primers for a variable light chain sequence are set out in Sequence ID Nos. 19 and 20.

The amplified fragments can be subcloned into plasmids, such as pUC19. Ideally, the amplified DNA fragment should include a promoter. Promoter elements, leader, and other sequences such as enhancer elements upstream or downstream

from the antibody peptides can be separately isolated and cloned into the plasmid containing the antibody encoding sequences.

A similar approach can be taken to isolate and subclone the sequences encoding the constant regions of the heavy and light chains that originate from another mammalian species. The enhancers to the heavy and light chain can be included in the isolated heavy chain fragments, or can alternatively be isolated and subcloned.

Human constant region DNA sequences are preferably isolated from immortalized B-cells, see e.g., Heiter et al., *Cell*, 22:197-207 (1980), incorporated by reference herein, but can be isolated or synthesized from a variety of other sources. The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene is described in Ellison et al., *Nucl. Acid. Res.*, 10:4071 (1982); Beidler et al., *J. Immunol.*, 141:4053 (1988); Liu et al., *Proc. Natl. Acad. Sci. USA*, 84:3439 (1987) (all incorporated by reference herein).

The CDRs for producing the immunoglobulins of the present invention preferably are derived from monoclonal antibodies capable of binding to the desired antigen, c-erbB-2 protein, and produced in any convenient mammalian source, including, mice, rats, rabbits, hamsters, or other vertebrate host cells capable of producing antibodies by well known methods. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("ATCC") ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the chimeric antibody peptides specifically described herein, other "substantially homologous" modified

immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques known to those skilled in the art. Modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, *Gene*, 8:81-97 (1979) and Roberts, S., et al, *Nature*, 328:731-734 (1987), both of which are incorporated herein by reference). Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess binding and/or effector activities. Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes to produce fusion proteins (e.g., immunotoxins) having novel properties or novel combinations of properties.

The cloned variable and constant regions can be isolated from plasmids and ligated together into a mammalian expression vector, such as pSV2-neo, and pRSV-gpt, to form a functional transcription unit. These expression vectors can then be transfected into host cells. Mouse myeloma cells, such as SP 2/0 or P3X cells, are a preferred host because they do not secrete endogenous immunoglobulin protein and contain all of the components used in immunoglobulin expression. Myeloma cells can be transfected using appropriate techniques as described above.

Other types of promoters and enhancers specific for other host cells are known in the art. See, Kameyoma et al., *supra*. For example, the DNA sequence encoding the chimeric antibody amino acid sequence can be linked to yeast promoters and enhancers and transfected into yeast by methods well known in the art. See, Kriegler, *supra*.

This same approach can be tak n to isolate the c-erbB-2 specific CDRs from one source such as one mammalian species and the framework regions of another source, such as a different mammalian species. The CDRs can then be ligated to the framework regions and constant regions to form a chimeric antibody. See, PCT No. GB88/00731 (1989), which is incorporated by reference. For example, the CDRs for the heavy chain of TAb 250, may be found within the variable region at the following amino acid positions on Sequence ID. No. 3: 31-35 (CDR1), 50-65(CDR2), and 98-105 (CDR3). The CDRs for the light chain may be found within the variable region at the following amino acid positions on Sequence ID. No. 4: 24-34 (CDR1), 50-56 (CDR2), and 89-97 (CDR3). The CDRs could be cloned in an expression vector comprising, for example, human framework and constant regions.

Another example is a recombinant DNA sequence comprising the heavy and/or light chain CDR1, CDR2, and CDR3 of one species, such as mouse, and the framework regions of human heavy chain to encode an antibody specific for c-erbB-2. Other possibilities include using CDRs specific for c-erbB-2; using part of the variable region encompassing CDR1 and CDR2 from one mammalian species, and then ligating this sequence to another encoding the framework portions of a second mammalian species to the CDR3 of the first; or transfecting a host cell line with a recombinant DNA sequence encoding a c-erbB-2 specific heavy chain CDRs derived from a first mammalian species, interspersed within the framework of a second mammalian species with a light chain containing a variable region DNA sequence derived from the first species and the constant region derived from the second species.

In one preferred embodiment, antibody peptides are comprised of the V_H, amino acid Sequence ID No. 3, and the V_K, Sequence ID No. 4, which are derived from the murine TAb 250

antibody. The TAb 250 variable regions obtained from its rearranged configuration in the myeloma's genome have the DNA coding strand set forth in Sequence ID No. 1, base positions 312-597 (V_H) and in Sequence ID No. 2 base positions 370-659 (V_K). The TAb 250 V_H D region is found at base positions 600-609 in Sequence ID No. 1, the J4 region at base positions 612-659 and the enhancer at base positions 1566-1813. The TAb 250 V_H sequence is linked to a human γ1 constant region. The TAb 250 V_K sequence is linked to a human κ constant region, and murine κ enhancer. The TAb 250 J2 region is found at base positions 659-691 of Sequence ID No. 2. Recombinant DNA expression vectors comprising these TAb 250 sequences may be transfected by electroporation into host cells. Standard selection procedures are used to isolate clones that produce the c-erbB-2 specific chimeric antibody.

Antibodies may be expressed in an appropriate folded form, including single chain antibodies, from bacteria such as *E. coli*. See, Pluckthun, *Biotechnology*, 9:545 (1991); Huse et al., *Science*, 246:1275 (1989) and Ward, et al., *Nature*, 341:544 (1989), all incorporated by reference herein.

The antibody peptide sequences may be amplified for cloning by use of polymerase chain reaction, or PCR, a technique used to amplify a DNA sequence of interest using a thermostable DNA polymerase, such as Taq polymerase, and polymerase and oligonucleotide primers, all as described in *PCR Protocols*, ed. Innis, et al., Academic Press, Inc. (1990), incorporated by reference herein. See also Orlandi, *supra* and Larrick et al., *Biotechnology*, 7:934 (1989), incorporated by reference herein.

A primer is an oligonucleotide which is capable of acting as a point of initiation of synthesis when placed under conditions typically which permit synthesis of a primer extension product which is complementary to a nucleic acid strand.

These conditions typically include the presence of four different nucleoside triphosphates in an appropriate buffer of proper ionic strength, pH, cofactors, etc., and suitable temperature. The oligonucleotide can be derived from a natural source, as a purified restriction fragment, or can be produced synthetically. PCR primers typically are preferably single stranded oligodeoxyribonucleotides, about 15-30 residues in length. The primers are substantially complementary to the sequences to be amplified such that they can hybridize to the target sites.

Primers are chosen such that one primer hybridizes to the 5' end of sequence of interest, and a second primer hybridizes to the 3' end of the sequence, but to the opposite strand.

In the first step of PCR, the reaction mixture is generally heated at about 90-100°C to denature the DNA. The primers hybridize with the target sequence, typically, at about 40-60°C for about 10-60 seconds, followed by extension of the primers, for example, at about 65-75°C for approximately 1 minute for every kb of DNA to be amplified. The products of synthesis become targets of the second primer, and a second cycle of hybridization and synthesis. The cycles are repeated about 25-40 times, typically in an automated temperature cycling machine. A sequence can be amplified 10^6 times or more using PCR.

Although polymerase chain reaction is a powerful cloning technique, problems can arise in choosing primers and establishing hybridization and extension conditions. Choosing an effective primer is often difficult. For example, a primer ideally has a high GC content, to optimize its ability to hybridize under high temperature conditions. Even more problematic and less predictable is the secondary structure of the primer or the complementary DNA. If either is prone, for example, to stem-loop formation, the primer might be unable to

hybridize to the DNA. Small amounts of contaminating DNA can result in the incorrect target being amplified.

The subject peptides may be used to make fusion proteins such as immunotoxins. Immunotoxins are characterized by two functional components and are particularly useful for killing selected cells in vitro or in vivo. One functional component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second functional component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known within the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., *Monoclonal Antibodies in Clinical Medicine*, Academic Press, pp. 168-190 (1982) and Waldmann, *Science*, 252:1657 (1991), both of which are incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, *Pseudomonas* exotoxin A, ricin, diphtheria toxin, ricin A chain, gelonin, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See, generally, "Chimeric Toxins," Olsnes and Phil, *Pharmac. Ther.*, 25:355-381 (1982), and "Monoclonal Antibodies

for Cancer Detection and Therapy," ds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), which are both incorporated herein by reference.)

The delivery component of the immunotoxin will include the peptides of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

For diagnostic purposes, the antibody peptides may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the first antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Anti-idiotypic antibodies to c-erbB-2 protein may also be produced using the peptides of this invention by immunizing a host with the peptides, including one or more of the CDRs and then immortalizing cells which express nucleic acid sequences that encode antibodies or idiotopic regions thereof. The immortalization process may be carried out by hybridoma fusion techniques, by viral transformation of human antibody-producing lymphocytes, or by techniques that combine cell fusion and viral transformation methodologies.

Monoclonal anti-idiotype antibodies may be prepared using a combination of Epstein-Barr virus (EBV) transformation and hybridoma fusion techniques such as those described by Kozbor et al., *Proc. Natl. Acad. Sci.* (1982) 79:6651, which is

incorporated herein by reference. For instance, the hybridomas may be created by fusing stimulated B cells, obtained from a human immunized with the primary (idiotype) antibody (Ab1) to which the anti-idiotype (Ab2) is to be made, with a mouse/human heterohybrid fusion partner. A variety of such fusion partners have been described. See, for example, James and Bell, *J. Immunol. Meths.* (1987) 100:5-104 and U.S. Patent No. 4,624,921, which are incorporated herein by reference. A mouse/human fusion partner may be constructed by fusing human lymphocytes stimulated or transformed by EBV with readily available mouse myeloma lines such as NS-1 or P3NS-1, in the presence of polyethylene glycol, for instance. The hybrid should be suitably drug-marked, which may be accomplished by cultivation of the hybrid in increasing concentrations of the desired drug, such as 6-thioguanine, ouabain, or neomycin.

Anti-idiotype antibodies of interest may also be accomplished using EBV transformation techniques. For example, B-lymphocytes are derived from peripheral blood, bone marrow, lymph nodes, tonsils, etc., of patients immunized with the idiotype antibody, and these lymphocytes are immortalized using EBV according to methods such as those described in U.S. Patent No. 4,464,465 and Chan et al., *J. Immunol.* (1986) 136:106, which are incorporated herein by reference.

The hybridomas or lymphoblastoid cells which secrete anti-idiotypic antibody of interest may be identified by screening culture supernatants against antibody which is specific for c-erbB-2. Cells from wells possessing the desired activity are cloned and subcloned in accordance with conventional techniques and monitored until stable immortalized lines producing the monoclonal antibody of interest are identified. The monoclonal antibodies thus produced may be of the IgG, IgM, IgA or IgD isotype, and may further be of any of

the subclasses of IgG, such as IgG_1, IgG_2, IgG_3, or IgG_4. Once an immortalized cell line is acquired, the antibodies can be used as a gene source for production of chimeric antibody peptides as described above.

Anti-idiotypic antibodies which act as internal images of a tumor antigen may be used to prime a *de novo* response to the c-erbB-2 protein. By presenting these images of antigenic epitopes in a different molecular environment, responses may be activated which would otherwise be silent. Nisonoff and Lamoyi, *Clin. Immunol. Immunopathol.* (1981) 21:397. That is, when the anti-idiotype represents a conformational image of the antigen, it may substitute for nominal antigen and elicit a primary antibody-like response. Anti-idiotypic antibodies which do not bear the internal image of antigen may also induce antitumor responses by influencing the regulatory idiotypic network. See, Bona, 1984, in *Idiotypy in Biology and Medicine*, Kohler et al., eds., Academic Press, pp. 29-42. Thus, antibodies to framework-associated idiotopes, or regulatory idiotopes, may select or amplify T and/or B cell clones with specificity for tumor antigens. Some evidence, however, suggests that this group of anti-idiotypic antibodies can prime a humoral response but are unable to cause maturation of B cells without further challenge with the nominal antigen (Heyman et al., *J. Exp. Med.* (1982) 155:994), and thus combination with an internal image anti-idiotypic antibody may be necessary to evoke a desired antitumor response.

Also contemplated here are those compounds that have designed specificities based upon the CDRs specific to c-erbB-2 protein, such as those described here. Organic compounds may be synthesized with similar biological activity by first determining the relevant contact residues and conformation involved in c-erbB-2 binding by an antibody peptide of this invention. Computer programs to create models of proteins

such as antibodies are generally available and well known to those skilled in the art (see, Kabat et al. *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, National Institutes of Health (1987); Loew et al., *Int. J. Quant. Chem., Quant. Biol. Symp.*, 15:55-66 (1988); Bruccoleri et al., *Nature*, 335:564-568 (1988); Chothia et al., *Science*, 233:755-758 (1986), all of which are incorporated herein by reference. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin et al., *J. Mol. Graphics*, 6:13-27 (1988)). For example computer models can predict charged amino acid residues that were accessible and relevant in binding and then conformationally restricted organic molecules can be synthesized. See, for example, Saragovi et al., *Science*, 253:792 (1991).

Other General Definitions.

"Recombinant" means that the subject product is the result of the manipulation of genes into new or non-native combinations.

"Restriction endonucleases" and "restriction enzymes" refer to enzymes which cut double stranded DNA at or near a specific nucleotide recognition sequence.

Complementary DNA, "cDNA" refers to DNA that is derived from a messenger RNA sequence (mRNA), for example, using reverse transcriptase. Reverse transcriptase is an enzyme that polymerizes DNA using an RNA template.

"Transcriptional activating sequences" refer to DNA sequences, such as promoters and enhancers, that activate transcription of a gene. Such sequences, in a proper host, drive transcription of a correctly positioned DNA sequence encoding a peptide. For example, the κ chain promoter and κ

chain enhancer will promote transcription of a correctly positioned DNA sequence in a myeloma or hybridoma host. Other transcriptional regulator sequences will often be useful in analogous circumstances, for example, when deactivation may be desired.

"Coding strand sequence" is the region of a gene that encodes the amino acid sequence of a protein.

A "promoter" is a DNA sequence 5' of the protein coding sequences which affects transcriptional activity. RNA polymerase first binds to the promoter to initiate transcription of a gene.

An "enhancer" is a DNA sequence that can positively affect transcriptional efficiency. A preferred enhancer for the sequence encoding a heavy chain variable region of the antibodies described here is that found at base positions 1566 to 1813 on Sequence Listing ID No. 1.

A "vector" is a sequence of DNA, typically in plasmid or viral form, which is capable of replicating in a host. A vector can be used to transport or manipulate DNA sequences. An "expression vector" includes vectors which are capable of expressing DNA sequences contained therein, typically producing a protein product. The coding sequences are linked to other sequences capable of effecting their expression, such as promoters and enhancers. Expression vectors are capable of replicating in a host in episomal form; others can integrate into a host cell's chromosome. Ideally, the expression vectors have a selectable marker, for example, neomycin resistance, which permits the selection of cells containing the marker.

An "oligonucleotide" is a polymer molecule of two or more nucleotides including either deoxyribonucleotides or ribonucleotides.

"Host cells" refer to cells which are capable or have been transformed with a vector, typically an expression vector. A host cell can be prokaryotic or eukaryotic, including bacteria, insect, yeast and mammalian cells.

Pharmaceutical Applications.

The chimeric antibodies or antibody peptides of this invention can be used in pharmaceutical compositions in dosages that are cytotoxic to tumor cells. Tumors or cancers to be treated with the compositions of this invention are any tumors which express, or are suspected of expressing, the c-erbB-2 oncogene protein or have amplification of the c-erbB-2 gene. These tumors include, for example: breast, ovarian, bladder, prostate, stomach, lung and thyroid cancers. The c-erbB-2 protein is reported to be expressed in: solid tumors by, for example, Gutman et al., *Int. J. Cancer*, 44:802-805 (1989); in human adenocarcinomas (solid tumors) by Yokota et al., *The Lancet*, April 5, 1986, p.765; in gastric and esophageal carcinomas by Houldsworth et al., *Cancer Res.*, 50:6417-22 (1990); in neoplastic cervix, vulva and vagina by Berchuck et al., *Obstetrics. Gynecol. Surv.*, 76:381 (1990); in renal cell carcinoma by Weidner et al., *Cancer Res.*, 50:4504 (1990); in lung adenocarcinomas by Kern et al., *Cancer Res.*, 50:5184-5191 (1990) and Schneider et al., *Cancer Res.*, 49:4968-4971 (1989); in gastric cancer by Fukushige, et al., *Mol. and Cell. Biol.*, 6:955-958 (1986), Park et al., *Cancer Res.*, 49:6605 (1989); in breast and ovarian cancer by Slamon et al., *Science*, 244:707 (1989); by Berchuck et al., *Cancer Res.*, 50:4087 (1990); by Van de Vijver et al., *Mol. Cell Biol.*, 7:2019-2023 (1987), by Varley et al., *Oncogene*, 1:423-430 (1987), Bacus et al., *Am. J. Path.*, 137:103 (1990) all of which are incorporated by reference herein.

The compositions can be used in either pre- or post-operative treatment of cancer or both. The compositions herein are preferably administered to human patients via oral, intravenous or parenteral administrations and other systemic forms. The pharmaceutical formulations or compositions of this invention may be in the dosage form of solid, semi-solid, or liquid such as, e.g., tablets, pills, powders, capsules, gels, ointments, liquids, suspensions, aerosols or the like. Preferably the compositions are administered in unit dosage forms suitable for single administration of precise dosage amounts. The compositions may also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants; or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. Effective amounts of such diluent or carrier will be those amounts which are effective to obtain a pharmaceutically acceptable formulation in terms of solubility of components, or biological activity, etc. Generally a dosage level of from 1-500 mg/m^2 of body surface area may be used systemically for the antibody compositions, to be adjusted as needed depending upon other agents used. For example, the compositions may be administered with an anti-neoplastic agent. For pharmaceutical treatments and guidelines see generally, *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, Seventh Ed., ed. Gilman et al., MacMillan Publishing Company (1985) and *Remington's*

Pharmaceutical Science, Sixteenth Ed., Mack Publishing Co. (1982), all incorporated by reference herein.

The tumor cells that one wishes to kill or control the growth of are referred to as "target tumor cells." "Test tumor cells" are any tumor cells *in vitro* that express the c-erbB-2 protein.

Imaging agents comprising a radio- or other label attached to the peptides of this invention are also contemplated for *in vivo* detection of cells expressing the c-erbB-2 protein. Radiolabels may be any labels appropriate for gamma camera imaging, such as radioiodines (^{131}I or ^{123}I) or radiometals (^{111}In or ^{99m}Tc), for example. The peptide selectively binds to the cells expressing c-erbB-2 present in the patient, thereby concentrating the label in the area of the target cells. Alternatively, the peptides can be labeled with paramagnetic contrast agents, and detected by nuclear magnetic resonance methods. The labeled antibodies thus produce a detectable image of the tumor tissue.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

The entire disclosures of all applications, patents and publications, cited above and below, are hereby incorporated by reference.

E X A M P L E S

I. Cells, Vectors, Probes, and Primers

The hybridoma that produced monoclonal antibody TAb 250 was generated using c-erbB-2 transformed NIH 3T3 cells. Mouse myelomas P3x63-Ag8.653 (P3X) and SP 2/O, and human cell line ARH-77 (ATCC CRL 1621, human plasma cell leukemia peripheral blood) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). SKBR-3 cells and c-erb-2 transfected NIH 3T3 cells were provided by Dr. S. Aaronson (NIH, Bethesda, MD). Genomic DNA was prepared either using A.S.A.P. kit from Boehringer Mannheim according to the manufacturer's instructions or using protocol from *Current Protocols in Molecular Biology*, ed. Ausubel Green Publishing Associates and Wiley-Interscience (1988), incorporated by reference herein. Plasmid vectors pSV2neo, pRSV5gpt (pRSVgpt), pBR322 and pUC19 were obtained from ATCC and pIBI 21 was obtained from International Biochemical Inc. (New Haven, CT). The mouse heavy chain JH probe was a 1.0 Kb DNA fragment containing the JH_3 and JH_4 region, which was isolated from plasmid pJ3J4. The mouse light chain JK probe was a 1.8 kb DNA fragment containing the J region, which was isolated from plasmid pJKHB.1. Both pJ3J4 and pJKHB.1 were from Dr. J. Donald Capra (Southern Medical Center, University of Texas). The probes used for identifying human constant heavy and light chain genes were oligonucleotides, designed from coding sequences of both genes and synthesized on an Applied Biosystem 381A DNA synthesizer (Applied Biosystems Inc., Foster City, California). All the primers used in polymerase chain reactions (PCR) and nucleotide sequencings were synthesized on an Applied Biosystem 381A DNA synthesizer.

II. Production of Monoclonal Antibody TAb 250

Example 1

Preparation of c-erbB-2 Monoclonal Antibodies

Production and characterization of TAb 250, a monoclonal antibody to c-erbB-2 is described in Langton, et al., *Can. Res.*, 51:2593-2598 (1991), incorporated by reference herein. Balb/c mice were immunized intraperitoneally and subcutaneously with $2x10^6$-$1x10^7$ NIH3T3 cells transfected with the human c-erbB-2 oncogene, $NIH3T3_t$, (*Science*, 237:178-182) emulsified 1:1 volume/volume in complete Freund's adjuvant. Sera was collected every two weeks and tested for reactivity in an ELISA assay (described below) against formalin fixed NIH3T3 or fixed $NIH3T3_t$ cells. Animals with positive titers were boosted intraperitoneally or intravenously with cells in PBS, and animals were sacrificed 4 days later for fusion. Spleen cells were fused with P3-X63Ag8.653 myeloma cells at a ratio of 1:1 to 7.5:1 with PEG 4000, generally as described by the procedure of Kohler and Milstein (*Nature*, 256:495-497). Fused cells were gently washed and plated in 96-well plates at $1-4x10^6$ cells/ml in RPMI 1640 medium. Wells were fed with HAT medium 24 hours after the fusion and then every 3 days for 2-3 weeks. When colony formation was visible, after 10-14 days, the supernatants were tested for reactivity in the ELISA assay. Prospective clones demonstrating good growth were expanded into 24-well plates and rescreened 7-10 days later. Positive wells were then assayed for external domain reactivity against live NIH3T3 and $NIH3T3_t$ cells by flow sorting analysis. Clones which were positive both by ELISA assay and flow sorting analysis were recloned either by limiting dilution or by single cell deposition using a flow cytometer. Cells were diluted and deposited into 96-well plates in the presence or absence of spleen feeder cells. Wells demonstrating growth were retested by ELISA and recloned an

additional one to three times. Supernatants from hybridoma clones were tested for isotype and subisotype, reactivity to surface expressed p185 protein on NIH3T3$_t$ cells by flow sorting analysis, and immunoprecipitation of a labeled p185 protein from transfected cells. Positive hybridomas were grown in tissue and injected into pristane-primed Balb/c mice, Balb/c nude mice or IRCF$_1$ mice for ascites production. Monoclonal antibodies were purified from ascites fluid by HPLC using a Bakerbond ABx column. Purified TAb 250 antibodies were dialyzed against PBS and stored at -20°C. All purified antibodies were tested for isotype, subisotype, and contaminating isotypes by radial immunodiffusion. Cell surface staining of p185 expressing cell lines was detected and quantified by flow sorting analysis, ELISA assay against transfected and untransfected NIH3T3 cells, and radioimmunoprecipitation of p185 from labeled c-erbB-2 expressing cell lines. The antibodies did not cross-react with the closely related EGF-receptor protein as shown by the failure to precipitate a radiolabeled 170 Kd protein from radiolabeled A-431 cells, and they were analyzed by SDS-PAGE and gel densitometry (all purified proteins are >90% immunoglobulin).

III. Cloning Mouse V_H and V_K Genes from TAb 250

Two genomic DNA fragments, one containing TAb 250 heavy chain variable region (V_H) and the other containing TAb 250 light chain variable region (V_K), were both isolated from hybridoma genomic DNA by PCR cloning. The PCR for V_H cloning was performed with 100 µl of reaction mix containing 0.1 µg of TAb 250 genomic DNA; 10 mM Tris HCl (pH 8.4); 2.5 mM $MgCl_2$; 50 mM KCl; 100 µg/ml gelatin; 200 µM of each dATP, dCTP, dTTP and dGTP; 0.25 µM of each PCR primer and 2.5 units of Taq polymerase. The reaction was carried out in a DNA thermal cycler (Perkin-Elmer-Cetus) as described above for 40 cycles

with reaction cycle set up as follows: denaturation at 94°C for 20 seconds; annealing at 50°C for 20 seconds, extension at 72°C for 3 minutes and autoextensions for 15 seconds. The PCR for light chain cloning was performed using the same conditions as for V_H amplification described above except the $MgCl_2$ concentration was 1.5 mM. In both V_H and V_K cloning the PCR primers were designed to amplify the entire genes, including promoter elements, leaders and variable regions. The amplified V_H gene fragment also includes its own enhancer sequence. By using GCG sequence analysis software package version 7.0 (Devereux, et al., A Comprehensive Set of Sequence Analysis Programs for VAX, *Nuc. Acid Res.* 12:387-95 (1984), three degenerative 5' primers were designed, of which one was effective (Sequence ID No. 18), and one specific 3' primer (Sequence ID No. 17) was designed for V_H cloning; and six degenerative 5' primers, of which one was effective (Sequence ID No. 20), and one specific 3' primer (Sequence ID No. 19), were designed for V_K cloning.

For the 3' primer of the heavy chain, mouse heavy chain immunoglobulin sequences from GCG were compared to determine a site downstream from the variable region that would be suitable for primer design and hybridization. Since it would be advantageous to include the region containing the enhancer element, a sequence just 3' to the putative enhancer was chosen. The enhancer has been shown by others to lie within a 312 bp (base pair) Pst I - Eco RI fragment.

Mouse κ chain immunoglobulin sequences from GCG were compared to determine a site downstream from the variable region that would be suitable for primer design. Since the exact region of the κ chain enhancer in relationship to the variable region has not been determined, a sequence 1280-1307 bp downstream of J5 was chosen. This region contained a Hind

III site at the 3' end which could be used for cloning into an appropriate vector.

Mouse heavy and light chain immunoglobulin variable region sequences from GCG were compared to determine a region suitable for primer design that could be used in conjunction with the 3' primer for amplification of variable region sequences. Since the variable regions between immunoglobulin molecules are not identical, the sequences were analyzed for regions of homology that could be used for designing degenerate oligonucleotide primers. Approximately 500 files containing heavy chain variable region sequences and 500 files containing light chain variable region sequences were analyzed for homology. Three areas were examined: the 5' end of the sequences encoding the mature immunoglobulin molecule, the 5' end of leader sequences, and sequences surrounding putative promoter elements upstream of the leader. Since it was anticipated that expression of recombinant antibody would be attempted in murine myeloma cells, use of the endogenous murine immunoglobulin promoter was thought to be advantageous. For the heavy chain, there were 21 files that included promoter element sequences. From extensively examining these sequences, it was found that they fell into one of three roughly homologous groups. Therefore, it was decided to synthesize three degenerate oligonucleotides as possible 5' primers. For the light chain, there were also 21 files with sequence information surrounding the promoter. These sequences fell into six groups with 2 sequences showing only limited homology to the others. Six degenerate oligonucleotides were synthesized for use as 5' primers to amplify light chain variable region sequences.

All primer sequences were run on a computer program to test for secondary structure. In addition, the 5' primers were compared to the 3' primer by a computer program to

eliminate the possibility f primer dimers. All primers were synthesized on an Applied Biosystems 381A DNA synthesizer and purified over an OPC column (Applied Biosystems).

The standard reaction conditions for PCR amplification from genomic DNA is described as follows: 100 µl of reaction mixture containing 0.1 µg of genomic DNA; 10 mM Tris-HCl (pH 8.4); 2.5 mM $MgCl_2$; 50 mM KCl; 100 µg/ml gelatin; 200 µM of each dATP, dCTP, dTTP and dGTP; 0.25 µM of each PCR primer and 2.5 units of TAQ polymerase. To obtain optimal PCR protocol for V_H and V_K cloning, the primer concentration, magnesium concentration, primer annealing temperature and reaction cycles were varied in the reactions. For V_H amplification, the following parameters were tested: primer concentration- 0.125 mM, 0.25 mM and 0.5 mM; magnesium concentration- 0.5 mM, 1.5 mM, 2.5 mM and 5.0 mM; TAQ polymerase- 1.25 units (u), 2.5 units, 5.0 units and 7.5 units; primer annealing temperature- 32°C, 40°C and 50°C; reaction cycles- 30 and 40. Over 30 PCR amplification protocols at various conditions were performed in order to achieve the amplification of V_H. For V_K amplification, the parameters tested were: magnesium concentration- 0.5 mM, 1.5 mM, 2.5 mM and 5.0 mM; primer annealing temperature- 50°C and 55°C, and reaction cycles- 35 and 40. Over 40 PCR amplification protocols were performed to achieve the V_K amplification.

Southern hybridizations with JH and JL probes were performed to verify that the PCR amplified fragments were immunoglobulin heavy and light chain variable regions. The PCR cloned TAb 250 V_H and V_K genes were subsequently subcloned into plasmid vector pUC19 at the Eco RI site (Figure 1) and pIBI21 at the Hind III site (Figure 2) respectively, and their nucleotide sequences were determined by plasmid DNA sequencing from both DNA strands. The mouse light chain enhancer, which

was not included in the cloned V_κ gene fragment, was cloned separately from TAb 250 genomic DNA by PCR.

The mouse κ enhancer was amplified from TAb 250 genomic DNA using PCR (Figure 3). The primers used are found in Sequence ID No. 9 (5' primer) and Sequence ID No. 10 (3' primer). Reaction conditions were the same as for the human γ1 constant region described below, except that 1mM $MgCl_2$ was used. Amplification was performed in a Perkin-Elmer Cetus (Norwalk, CT) instrument with the following procedure: denature 95°C, 30 seconds, anneal 55°C, 30 seconds, extend 72°C, 90 seconds, for thirty cycles. The amplified DNA was extracted with chloroform, and primers were removed using Geneclean (BIO 101 Inc., La Jolla, California). The DNA was digested with Eco RI, run on an agarose gel, excised, and purified using Geneclean. Plasmid pUC19 was digested with Eco RI and treated with calf intestine alkaline phosphatase. The κ enhancer DNA was then cloned into pUC19 to create plasmid p19enhancer. The κ enhancer sequence was verified by restriction mapping.

Attached as Appendix A is a sequence map of the heavy chain variable region of TAb250, with the location of such region indicated by the symbol VH. A promoter element, leader, D region, J4 region and enhancer region are also indicated. Attached as Appendix B is a sequence map of the light chain variable region of TAb250, with the location of such region indicated by the symbol vk. A promoter element, leader and J2 regions are also indicated.

IV. Cloning Human Cγ1 and Cκ Genes

The human heavy chain γ1 constant (Cγ1) gene and the human light chain κ (C_κ) gene were cloned from human IgG producing cell line ARH-77 (ATCC CRL 1621) by using the similar PCR approach as described above and were verified by

Southern hybridization using oligonucleotide probes to Cγ1 and Cκ coding region. Each amplified Cγ1 and Cκ gene fragment contained several hundred base pair flanking sequences at both the 5' and 3' end.

A. The γ1 constant region was amplified from ARH-77 genomic DNA using PCR. The primers used are found in Sequence ID No. 5 (5' primer) and No. 6 (3' primer). Reaction conditions were 10 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 10 μg/ml gelatin, 0.25 μM each PCR primer, 0.2 mM dNTPs, 2 ng/μl genomic DNA, 0.025 u/μl TAQ polymerase in 100 μl total volume. Amplification was performed in an ERICOMP, Inc. (San Diego, California) instrument with the following procedure: denature at 95°C, for 30 seconds, anneal at 55°C, for 30 seconds, and extend 72°C for 150 seconds, for thirty cycles. The amplified DNA was verified to be the γ1 constant region by Southern blot (see Sequence ID No. 11 for probe) and sequence analysis. The amplified DNA was extracted with chloroform and primers were removed using Geneclean (BIO 101) according to the manufacturer's directions. The DNA was digested with Bam HI and Sal I (New England Biolabs, Beverly, Massachusetts) according to the manufacturer's directions. The DNA was then run on an agarose gel, excised, and purified using Geneclean. Plasmid pBR322 was digested with Bam HI and Sal I and treated with calf intestine alkaline phosphatase (Boehringer Mannheim Biochemical, Indianapolis, Indiana). The γ1 PCR DNA was then cloned into pBR322 to create plasmid 322γ1 (Figure 4).

B. The κ constant region was amplified from ARH-77 genomic DNA using PCR. The primers used are found in Sequence ID No. 7 (5' primer) and No. 8 (3' primer). Reaction conditions were the same as above, except that 2 mM $MgCl_2$ was used. Amplification was performed in ERICOMP with the following procedure: 10 cycles: denature at 94°C, for 30 seconds, anneal at 55°C, for 30 seconds, and extend at 72°C,

for 150 seconds; followed by 10 cycles which were the same except that extension occurred for 210 seconds; followed by 10 cycles which were the same except that extension occurred for 270 seconds. The amplified DNA was verified to be the human κ constant region by Southern blot. (See Sequence ID No. 12 for probe.) The amplified DNA was extracted with chloroform, and primers were removed using Geneclean. The DNA was digested with Bam HI and Eco RI, and purified using Geneclean. pUC19 was digested with Bam HI and Eco RI and treated with calf intestine alkaline phosphatase. The κ PCR DNA was then cloned into pUC19 to create $p19_K$ (Figure 5).

V. Construction of Expression Plasmids

A. Construction of plasmid SVNH-L (chimeric heavy chain expression plasmid)

1) The mouse heavy chain variable region (V_H) was subcloned into pBR322: pUC $19V_H$ was digested with Eco RI and Sal I, the fragment was gel isolated and subcloned into pBR322 Bam HI/Sal I with the use of an oligonucleotide adapter (see Sequence ID Nos. 13 and 14) to create plasmid $322V_H$ (Figure 6).

2) The mouse V_H and the human γ1 constant region were subcloned into pSV2neo: $p322V_H$ was digested with Bam HI and Sal I, and the V_H fragment was gel isolated. p322γ1 was digested with Bam HI and Sal I, and the Cγ1 fragment was gel isolated. Plasmid pSV2neo was digested with Bam HI and treated with calf intestine alkaline phosphatase. The V_H and Cγ1 fragments were ligated into pSV2neo to create plasmid SVNH-L (Figure 7).

B. Construction of plasmid RGL-L (chimeric light chain expression plasmid)

1) The mouse light chain variable region (Vκ) was subcloned into pBR322: pIBI21V_κ was digested with Hind III, the V_κ fragment was gel isolated and subcloned into pBR322 which had been digested with Hind III and treated with calf intestine alkaline phosphatase to create plasmid 322V_κ (Figure 8).

2) The mouse V_κ and the human κ constant region (C_κ) were subcloned into p322ΔRH: p322V_κ was digested with Bam HI and Eco RI and the V_κ fragment was gel isolated. Plasmid p19κ was digested with Bam HI and Eco RI and the C_κ fragment was gel isolated (Figure 9). Plasmid p322ΔRH was digested with Bam HI and treated with calf intestine alkaline phosphatase. The V_κ and C_κ fragments were ligated into p322ΔRH to create plasmid ΔRH-light (Figure 10).

3) A portion of the intron 3' to the V_κ region was deleted from pΔRH-light: pΔRH-light was digested with Eco RI and Xba I and the vector fragment was gel isolated. The Eco RI/Xba I oligonucleotide adapter (Sequence ID Nos. 15 and 16) was ligated into the vector to create plasmid Δ-light (Figure 11).

4) The mouse κ enhancer (cloned using primers of Sequence ID Nos. 9 and 10) was added to pΔ-light: p19enhancer was digested with Eco RI and the enhancer fragment was gel isolated. pΔ-light was digested with Eco RI and treated with calf intestine alkaline phosphatase. The enhancer fragment was ligated into pΔ-light to create pΔL-enhancer (Figure 12).

5) The light chain fragment containing V_κ, κ constant and enhancer regions from pΔL-enhancer was subcloned into pRSV-gpt: pΔL-enhancer was digested with Bam HI and the light chain fragment was gel isolated. pRSV-gpt was digested with Bam HI and treated with calf intestine alkaline phosphatase.

The light chain fragment was ligated into pRSV-gpt to create plasmid RGL-L (Figure 13).

C. Gene Transfection

The chimeric heavy chain plasmid and the chimeric light chain plasmid were cut by restriction enzyme Pvu I and Bgl I respectively in a non-essential region of the plasmid. Samples of 5 μg to 50 μg of linear heavy and light chain plasmid DNA were cotransfected into $1x10^7$ SP 2/0 cells in 1 ml PBS (phosphate buffered saline) by electroporation at 300 volts and 800 μF using cell-porator electroporation system (Bethesda Research Laboratories, Inc., Gaithersburg, MD, "BRL"). The electroporated cells were recovered in RPMI medium (BRL) supplemented with 10% fetal calf serum under 5% CO_2 at 37°C for 24-48 hours. The cells were then dispensed into 48-well tissue culture plates at $1x10^4$ cells/well under G418 antibiotic selection at concentration of 300 μg/ml.

IV. ELISA Analysis of Chimeric Antibody Expression

Culture supernatants harvested from G418 resistant transfectants were screened for chimeric heavy chain and chimeric light chain expression by a two antibody sandwich ELISA. Human IgG (Sigma Chemical Co., St. Louis, MO) was used as a standard for both heavy and light chain ELISA, prepared at concentrations ranging from 10 ng/ml to 1 μg/ml. Ninety-six well plates were coated with 100 μl of anti-human IgG_1 monoclonal antibody (Sigma) diluted 1:400 (v/v) in PBS or 100 μl of 2 μg/ml goat anti-human κ light chain (Sigma) at 4°C for 15-18 hours. Plates were washed once with 0.05% Tween 20 in PBS and blocked with 200 μl of 2% BSA in PBS for 1 hour. After washing with PBS, 100 μl of culture supernatants or human IgG standards were added to the wells and incubated for 3 hours at room temperature. Plates were then washed six

times with PBS and 100 µl of goat anti-human IgG γ chain specific HRP (horseradish peroxidase) conjugate (Zymed Lab Inc., South San Francisco, California) diluted at 1:2000 or goat anti-human IgG (H+L) - HRP conjugate (Zymed Lab Inc.) diluted at 1:2000 was added to each well. Incubation was carried on for 1 hour at room temperature. Plates were washed six times in 0.05% Tween 20 in PBS and developed by adding 100 µl of TMB/peroxidase substrate at 1:1 (v/v) (Kirkegaard and Perry Lab Inc., Gaithersburg, Maryland). The reaction was terminated by adding 50 µl of 1 M phosphoric acid and the absorbance measured at a dual wave length of 450nm/595nm on a microplate reader equipped with analysis software package (Molecular Devices Corp.). To measure the amount of antibody secreted by the transfected clone, $1x10^6$ cells were washed with selection medium (RPMI supplemented with 10% fetal calf serum; 2 mM Glutamine; 1 mM sodium pyruvate and 300 µg/ml G418) and resuspended in 1 ml of the same medium. After 48 hours at 37°C, the supernatants were recovered, a serial dilution of the culture supernatants was prepared and samples were assayed by ELISA as described above.

V. Characterization of Chimeric Antibody by Cell Labeling and Immunoprecipitation

An aliquot of $1x10^7$ cells was pelleted and washed twice in methionine and cysteine free RPMI medium. The cell pellet was resuspended in 3 ml of the same methionine and cysteine free medium and labeled with 300 µCi of Trans ^{35}S-label ^{35}S (^{35}S-methionine, ^{35}S-cysteine; ICN) for 6 hours at 37°C under 5% CO_2 with occasional swirling. Cell supernatants were collected for analysis of secreted antibody. Cell pellets were lysed in RIPA buffer (50 mM Tris-HCl pH 7.5; 150 mM NaCl; 1% sodium deoxycholate; 1% NP-40 and 0.1% SDS) and following centrifugation at 100,000 xg for 30 minutes, supernatants were

collected for analyzing cytoplasmic antibody. Goat anti-human κ light chain antibody (Sigma) was used in the immunoprecipitation. After the culture supernatants or cell lysates were incubated with 10 μg of the antibody for 2 hours at 4°C, 50 μl of slurry of protein A sepharose CL-4B (Pharmacia LKB Biotechnology, Inc., New Jersey) was added and incubated for another 30 minutes. The antibody bound protein A beads were pelleted in a microfuge by a 30 second centrifugation, washed twice each in high salt (1 M NaCl) RIPA buffer and RIPA buffer, and resuspended in Laemmli sample buffer. The samples were analyzed directly under non-reducing conditions on a 4-20% SDS polyacrylamide gradient gel or under reducing conditions (by including 0.15 M mercaptoethanol in the sample buffer) on a 12% SDS polyacrylamide gel. The gels were fixed, treated with the autoradiography enhancer, Amplify (Amersham), dried and exposed to Kodak XAR-5 film.

A. Binding Assays of Chimeric Antibody

The binding activity of TAb 250 chimeric antibody, designated BACh 250, was tested by EIA and competition binding assay. EIA was performed in 96-well microtiter plates coated with glutaraldehyde fixed c-erbB-2 transfected NIH 3T3 cells at $1x10^4$ cells per well. Culture supernatants at serial dilutions were added and incubated for 3 hours at room temperature. The unbound antibody was removed by two PBS washes. Goat anti-human IgG (H+L)-horseradish peroxidase conjugate (Zymed Lab Inc.) was added and incubated for two hours at room temperature. The plate was developed and analyzed as described for the ELISA.

B. ^{125}I-TAb 250 Competition Assay

TAb 250 and supernatants from BACh 250 secreting clones E8 and A7 were tested for their ability to influence the

binding of ^{125}I-TAb 250. TAb 250 was radiolabeled using Iodobeads (Pierce Chemical Company, Rockford, Illinois) according to the manufacturer's specifications. Carrier-free Na^{125}I (400 μCi of IMS.30, Amersham Corporation, Arlington Heights, Illinois) was reacted with 25 μg TAb 250 in 100 mM Na-phosphate buffer (200 μl, pH 7.4) in the presence of 3 Iodobeads. This resulted in an approximate ratio of one iodine atom per IgG molecule. The incorporation was allowed to proceed at room temperature for 7.5 minutes with intermittent mixing. The reaction mixture was removed from the beads, and after 5 minutes, the volume was adjusted to 0.5 ml with Na-phosphate buffer and 2 μl were taken to estimate specific activity (see below). The remaining volume was de-salted by gel filtration using a NAP-5 column (Pharmacia) equilibrated with PBS containing 0.1% BSA and 0.02% azide. The radiolabeled antibody was eluted in 1 ml column buffer and was stored at 4°C for up to 6 weeks with no apparent loss of binding activity. The de-salted material was essentially free of unincorporated iodine since >95% was TCA-precipitable.

The specific activity of the radiolabeled antibody was estimated by TCA precipitation of the material before the de-salting step. Thus, 2 μl of the reaction mixture was diluted 500-fold in column buffer and duplicate aliquots mixed with an equal volume of ice-cold 20% TCA. After 15 minutes on ice the precipitated material was collected by centrifugation (10 min, 3000 xg). Supernatants and pellets were counted separately, and the incorporation was expressed as the percent of TCA-precipitable counts. The incorporation obtained in separate iodinations ranged from 27% to 45%, yielding specific activity estimates from 3.9 to 7.2 μCi/μg. Before each binding experiment, an appropriate amount of ^{125}I-TAb 250 was de-salted by gel filtration using a NAP-5 column equilibrated in binding

buffer. This procedure removed the azide and yielded material that was routinely >98% TCA-precipitable.

Single cell suspensions of SKBR3 cells and ^{125}I-labeled TAb 250 were prepared as previously described in commonly assigned U.S.S.N. 07/644,361. Sample dilutions were prepared in binding buffer (MEM medium supplemented with 0.1% BSA, 50 mM HEPES pH 7.0). Samples of culture supernatants or cold TAb 250 prepared at various concentrations were incubated with 10 µl of ^{125}I-labeled TAb 250 at specific activity of 8 x 10^5 cpm/µg, and 1x10^4 cells of prepared SKBR3 cells in a final volume of 100 µl, on ice in a shaker at 80 rpm for 4 hours. To terminate the reaction, 800 µl of ice-cold binding buffer was added to reaction mix and the supernatant was removed by centrifugation. The cell associated radioactivity was measured by counting the cell pellets in an Isodata γ counter. As shown in Figure 14, the BACh 250 antibodies displaced ^{125}I-TAb 250 binding in a manner comparable to unlabeled TAb 250.

C. Effect of BACh 250 on Cell Proliferation

The chimeric anti-c-erbB-2 antibody BACh 250 effect on cell proliferation was tested on the c-erbB-2 bearing tumor cell line SKOV3. SKOV3 cells were seeded in growth medium into 24-well dishes at 10,000 cells/well. After 24 hours at 37°C, monoclonal antibodies to c-erbB-2, TAb 250, BACh250, or purified Fab or $F(ab')_2$ fragments of TAb 250 were added to yield a final assay concentration of 10 µg/ml. At various times after the addition of antibodies, the cells were removed with trypsin and quantified using a Coulter Counter. In addition, representative cell samples were stained with propidium iodide and analyzed using a FACS Scan (Becton-Dickinson, Mountain view, California) to determine the percentage of viable cells in each treatment group. Each point represents

the mean of triplicates and is expressed as a percentage of the viable cell number compared to untreated control wells.

TAb 250 and BACh 250 had a similar effect on proliferation. The proliferation of SKOV3 cells was suppressed to approximately 60% of control cell number by TAb 250 after 10 days, while proliferation of SKOV3 cells was suppressed to approximately 58% of control cell number by BACh 250. Treatment with $F(ab')_2$ fragments of TAb 250 reduced cell growth to 76% of control levels. While control cells were assessed to be >98% viable, cells treated with these antibodies demonstrated a small but significant loss of viability (from 84 to 89%).

D. Complement Fixation by BACh 250

TAb 250 or BACh 250 (3.1-25 μg/ml) was added to ^{51}Cr-labeled SKBR-3 cells (which express high levels of c-erbB-2), in the presence of rabbit complement. The cells were incubated at 37°C for 1 hour, and supernatants were harvested and counted in a gamma counter. The percent of specific ^{51}Cr release was calculated as the difference between experimental and background release divided by the difference between total release and background release. Total release was calculated by lysing cells in 10% SDS, and the background release was determined in the absence of complement.

While the chromium release in the presence of TAb 250 and rabbit complement remained at essentially background levels, 6.25 μg/ml of BACh 250 in the presence of 1:10 dilution of rabbit complement caused approximately 67% of ^{51}Cr release. See Figures 16A and B. These results indicate that BACh 250 can fix rabbit complement more effectively than TAb 250.

E. Antibody dependent cellular toxicity m diated by human effector cells

Human effector cells were isolated by density gradient centrifugation. The effector cells were added to ^{51}Cr-labeled SKBR-3 cells at various effector to target ratios (E:T) in the presence of IgG_1 control antibody, TAb 250, or BACh 250. Plates were incubated for 24 hours at 37°C. Supernatants were harvested and counted in a gamma counter. The specific chromium release was more than twice that of the IgG_1 at optimal E:T ratios.

F. In vivo effect of BACh 250

Female Balb\c nu\nu mice were implanted with SKOV-3 cells. Treatments were started 7 days after tumor cell implant. Animals were treated with either an IgG_1 isotype control monoclonal antibody, TAb 250, or BACh 250 at 1000 μg/dose. Treatments were given interperitoneally on a schedule of one time a week for three weeks. After 36 days, the tumors in the BACh 250 animals were approximately one-third the volume of the tumors from the control animal tumors, while the tumors in the TAb 250 treated animals were approximately two-thirds the volume of the tumors from the control animals.

In the same experiment when TAb 250 or BACh 250 were combined with cisplatin, both were effective in reducing tumor size. After 36 days, tumors from the treated groups were approximately one third the volume of IgG or cisplatin treated control groups.

In a second experiment, the combination treatment of BACh 250 and cisplatin inhibited tumor growth an average of 85%. This was similar to the inhibitory effects observed with TAb 250 and cisplatin. However, in the BACh 250 and cisplatin groups, 3 of 8 mice showed no tumor growth or tumor regression as compared to 0 of 8 mice showing regressions in the control

groups. One of eight mice showed no tumor growth in the TAb 250 plus cisplatin groups. Thus, the chimeric antibody appears to be as effective as the parental antibody from which it was derived, in inhibiting tumor growth when used above or in combination with chemotherapeutic drugs such as cisplatin.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

SEQUENCE LISTING

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
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(C) REFERENCE/DOCKET NUMBER: A120

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1824 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(D) OTHER INFORMATION: /label= CDR3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GACAGAAAAA TTGTTTGTTT TGTTAGTGAC AGTTTTCCAA CCAGCATTCT CTGTTTGCAG 300

GTGTCCAGTG TGAAGTGAAG CTGGTGGAGT CTGGGGGAGA CTTAGTGCAG CCTGGAGGGT 360

CCCTGAAACT CTCCTGTGCG ACCTCTGGAT TCTCTTTCAG TGACTTTTAC ATGTATTGGG 420

TTCGCCAGAC TCCAGAAAAG AGGCTGGAGT GGGTCGCATA TGTTAGTTCT GGAGGTGAGA 480

GCTATTATTC AGACACTATA AGGGGCCGAT TCACCTTCTC CAGAGACAGT GCCAAGAACA 540

CCCTGCACCT GCAAATGAGC CGTCTGAAGT CTGAGGACAC AGCCATGTAT TTCTGTGCAA 600

GATTTGGTGA CTCTGCTATG GACTACTGGG GTCAAGGAAC CTCAGTCACC GTCTCCTCAG 660

GTAAGAATGG CCTCTCCAGG TCTTTATCTT TACCCTTTGT TTTGGAGTTT TCTGAGCATT 720

GTAGACTATT CTTGGATATT TGTCCCTGAG GGAGCCGGCT GACAGAAGTT GGGAAATGAA 780

CTGTCTAGGG ATCTCAGAGC CTTTAGGGCA GATTATCTCC ACATCTTTGA AAAACTTAGA 840

ATCTGTGTGA TGGTGTTGGT GGAGTCCCTG GATGATGGGA TAGGGACTTT GGAGGCTCAT 900

TTGAGGGAGA TGCTAAAATA GTCCTATGGC TGGAGGGATA GTTGGGGCTG TAGTTGGAGA 960

TTTTCAGTTT TTAGAATAAA AGTATTAGCT GCGGAATACA CTTCAGACCA CCTCTGTGAC 1020

AGCATTTATA CAGTATGCAT AGGGACGTGG AGTGGGGCAC TTTCTTTAGA TTTGTGAGGA 1080

ATGTTCCACA CTAGATTGTT TAAAACTTCA TTTGTTGGAA GGAGAGCTGT CTTAGTGATT 1140

GAGTCAAGGG AGAAAGGCAT CTAGTCTCGG TCTCAAAAGG GTAGTTGCTG TCTAGAGAGG 1200

TCTGGTGGAG CCTGCAAAAG TCCAGCTTCA AAGGAACACA GAAGTATGTG TATGGAATAT 1260

TAGAAGATGT TGCTTTTACT CTTAAGTTGG TTCCTAGGAA AAATAGTTAA ATACTGTGAC 1320

TTTAAAATGT GAGAGGGTTT TCAAGTACTC ATTTTTTTAA ATGTCCAAAA TTTTTGTCAA 1380

TCAATTTGAG GTCTTGTTTG TGTAGAACTG ACATTACTTA AAGTTTAACC GAGGAATGGG 1440

AGTGAGGCTC TCTCATACCC TATTCAGAAC TGACTTTTAA CAATAATAAA TTAAGTTTAA 1500

AATATTTTTA AATGAATTGA GCAATGTTGA GTTGGAGTCA AGATGGCCGA TCAGAACCAG 1560

AACACCTGCA GCAGCTGGCA GGAAGCAGGT CATGTGGCAA GGCTATTTGG GGAAGGGAAA 1620

CCAGCCCCAC CAAACCGAAA GTCCAGGCTG AGCAAAACAC CACCTGGGTA ATTTGCATTT 1680

CTAAAATAAG TTGAGGATTC AGCCGAAACT GGAGAGGTCC TCTTTTAACT TATTGAGTTC 1740

AACCTTTTAA TTTTAGCTTG AGTAGTTCTA GTTTCCCCAA ACTTAAGTTT ATCGACTTCT 1800

AAAATGTATT TAGTCGACGA ATTC 1824

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1657 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 32..39
(D) OTHER INFORMATION: /label= promoter octamer

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 120..179

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 370..659
(D) OTHER INFORMATION: /label= vk

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 659..691
(D) OTHER INFORMATION: /label= J2

(ix) FEATURE:
(A) NAME/KEY: region
(B) LOCATION: 440..472
(C) OTHER INFORMATION: /label= CDR1

(ix) FEATURE:
(A) NAME/KEY: region
(B) LOCATION: 518..538
(D) OTHER INFORMATION: /label= CDR2

(ix) FEATURE:
(A) NAME/KEY: region
(B) LOCATION: 635..661
(D) OTHER INFORMATION: /label= CDR3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGCTTCATT TACTTCCTTA TTTGGTGACT ACTTTGCATA GATCCCTAGA GGCCAGCACA 60

GCTGCCCATG ATTTATAAAC CATGTCTTTG CAGTGAGATC TAAAATACAT CAGACCAGCA 120

TGGGCATCAA GATGGAGTTT CAGACCCAGG TCTTTGTATT CGTGTTGCTC TGGTTGTCTG 180

GTGAGACATT TAAAAGTATT ATAAAATCTT AAAAGTAATT TATTTAAATA GCTATTTCCT 240

ATAGGAAGCC AATATTAGGT CAGACAATAC CATTAAATAA GACATTTTGG ATTCCAACAA 300

TTGTATTATG AAGTCTTTGT ATATGTAAGT GTATAGTCAT TATCTATTTC TGATTGCAGG 360

TGTTGATGGA GACATTGTGA TGACCCAGTC TCAAAAATTC ACGTCCACAT CAGTAGGAGA 420

CAGGGTCAGC ATCACCTGCA AGGCCAGTCA GAATGTTCGC ACTGCTGTAG CCTGGTTTCA 480

ACAGAGACCA GGGCAGTCTC CTAAAGCGCT GATTTACTTG GCATCCAACC GGCACACTGG 540

AGTCCCTGAT CGCTTCACAG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATTAGCAA 600

TGTGCAATCT GAAGACCTGG CAGATTATTT CTGTCTGCAA CATTGGAATT ATCCTCTCAC 660

GTTCGGAGGG GGGACCAAGC TGGAAATAAA ACGTAAGTAG TCTTCTCAAC TCTTGTTCAC 720

TAAGTCTAAC CTTGTTGAGT TGTTCTTTGC TGTGTGTTTT TCTTAAGGAG ATTTCAGGGA 780

GTCAGCAAAT TCCATTCTCA GATCAGGTGT TAAGCAGGGA AAGCTGTCCC ACAAGAGTTT 840

GGAACGATTT TCAGGCTAAA TTTCAGGCTT CTAAAGCAAA GTGATTAAAG TAGGGGAAGA 900

GGGATAAATG TCTTCCTTGG GAGGGTTTTG AGGTGGTAAA GTTAAAATAA ATCACTGTAA 960

ATCACATTCA GTGATGGGAC CAGACTGGAA ATATAACCTA AGTACATTTT TGCTCAACTG 1020

CTTGTGAAGT TTTGGTCCCA TTGTGTCCTT TGTATGAGTT TGTGGTGTAC TTAGATAAAT 1080

GAACTATCCT TGTAACCCAA AACTTAAGTA GAAGAGAACC AAAAATCTAG CTACTGTTCA 1140

AGCTGAGCAA ACAGACTGAC CTCATGTCAG ATTTGTGGGA GAAAAGAGAA AGGAACAGTT 1200

TTTCTCTGAA CTTAGCCTAT CTAACTGGAT CAGCCTCAGG CAGGTTTTTG TAAAGGGGGG 1260

CGCAGTGATA TGAATCACTG TGATTCACGT TCGGCTCGGG GACAAAGTTG GAAATAAAAC 1320

GTAGGTTGAC TTTTGCTCAT TTACTTGTGA CGTTTTGCTT CTGTTTGGGT AACTTGTGTG 1380

ACTTTGTGAC ATTTTGGCTA AATGACCATT CCTGGCAACC TGTGCATCAT TAGAAGATCC 1440

CCCAGAAAAG AGTCAGTGTG AAAGCTGAGC GAAAAACTCG TCTTAGGCTT CTGAGACCAG 1500

TTTTGTAAGG GGAATGTAGA AGAGAGAGCT GGGCTTTTCC TCTGAATTTG GCCCATCTAG 1560

TTGGACTGGC TTCACAGGCA GGTTTTTTTA GAGAGGGACA TGTCATAGTC CTCACTGTGG 1620

CTCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAC 1657

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 31..35
(D) OTHER INFORMATION: /label= CDR1

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 50..65
(D) OTHER INFORMATION: /label= CDR2

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 98..105
(D) OTHER INFORMATION: /label= CDR3

(ix) FEATURE:
(B) LOCATION: 51
(D) OTHER INFORMATION: /label= Residue may be Val or Ile

(ix) FEATURE:
(B) LOCATION: 57
(D) OTHER INFORMATION: /label= Residue may be Asn or Ser

(ix) FEATURE:
(B) LOCATION: 69
(D) OTHER INFORMATION: /label= Residue may be Ile or Phe

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Lys Leu Asp Cys Ala Thr Ser Gly Phe Ser Phe Ser Asp Phe
            20                  25                  30
```

Tyr Met Tyr Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Tyr Xaa Ser Ser Gly Gly Glu Xaa Tyr Tyr Ser Asp Thr Ile Arg
50 55 60

Gly Arg Phe Thr Xaa Ser Arg Asp Ser Ala Lys Asn Thr Leu His Leu
65 70 75 80

Gln Met Ser Arg Leu Lys Ser Glu Asp Thr Ala Met Tyr Phe Cys Ala
85 90 95

Arg Phe Gly Asp Ser Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Lys Asn Gly Leu Ser Arg Ser Leu Ser Leu Pro
115 120 125

Phe Val Leu Glu Phe Ser Glu His Cys Arg Leu Phe Leu Asp Ile Cys
130 135 140

Pro
145

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 24..34
(D) OTHER INFORMATION: /label= CDR1

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 50..56
(D) OTHER INFORMATION: /label= CDR2

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 89..97
(D) OTHER INFORMATION: /label= CDR3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Thr Ser Thr Ser Val Gly
1               5                  10                  15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn Val Arg Thr Ala
            20                  25                  30

Val Ala Trp Phe Gln Gln Arg Pro Gly Gln Ser Pro Lys Ala Leu Ile
        35                  40                  45

Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65                  70                  75                  80

Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp Asn Tyr Pro Leu
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Lys
            100                 105
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "gamma-1 constant region 5' primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAAGTCGACA GCAGGTGCAC ACCCAATGCC CAT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..31
(D) OTHER INFORMATION: /note= "gamma-1 constant region 3' primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTAGGATCCA GAACCATCAC AGTCTCGCAG G 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "kappa constant region 5'primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGTGAATTCC TGTCTGTCCC TAACATGCCC TGT 33

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "kappa constant region 3'primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTGGATCCT GACCGTAAGA CCTGTCACCC TTA 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "kappa enhancer 5'primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTAGAATTCA GCTTTTGTGT TTGACCCTTC CCT 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "kappa enhancer 3'primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGGAATTCA GCTAAACCTA CTGTATGGAC AGG 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..60
(D) OTHER INFORMATION: /note= "gamma oligo probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACGGTCACCA CGCTGCTGAG GGAGTAGAGT CCTGAGGACT GTAGGACAGC CGGGAAGGTG 60

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..60
(D) OTHER INFORMATION: /note= "kappa oligo probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTGAGGCT GTAGGTGCTG TCCTTGCTGT CCTGCTCTGT GACACTCTCC TGGGAGTTAC 60

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "BamHI/EcoRI adapter oligo"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCACTGG 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "BamHI/EcoRI adapter region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTCCAGTG 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "XbaI/EcoRI adapter oligo"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTAGACAGTG 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /note= "XbaI/EcoRI adapter oligo"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AATTCACTGT 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..37
(D) OTHER INFORMATION: /note= "3' Heavy Chain Variable Region PCR Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAGGAATTCG TCGACTAAAT ACATTTTAGA AGTCGAT 37

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..32
(D) OTHER INFORMATION: /note= "5' Heavy Chain Variable region PCR Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAGGAATTCM TATAGCAGRA MSAYATGCAA AT 32

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /note= "3' Light Chain Variable region PCR Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CATTAAGCTT TTAATATAAC ACTGGATA 28

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "5' Light Chain Variable Chain Region PCR Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

MAWTTACTTC CTTATTTGRT GACTRCTTTG CAT 33

WHAT IS CLAIMED IS:

1. A recombinant DNA sequence encoding at least one CDR region derived from an antibody specific for c-erbB-2 protein.

2. The sequence of claim 1, wherein the CDR region is derived from a heavy chain variable region of the antibody.

3. The sequence of claim 2, wherein the CDR region is a sequence selected from the group consisting of those that encode for the amino acid sequence at about position 31 to 35, 50 to 65, and 98 to 105 set forth on Sequence ID No. 3.

4. The sequence of claim 1, wherein the CDR region is derived from a light chain variable region of the antibody.

5. The sequence of claim 4, wherein the CDR region is a sequence selected from the group consisting essentially of those that encode for the amino acid sequence at about position 24 to 34, 50 to 56, and 89 to 97 set forth on Sequence ID No. 4.

6. The sequence of claim 1, wherein the antibody causes down modulation of c-erbB-2 protein or induces an increase in phosphorylation of c-erbB-2 protein when placed in contact with cells expressing the c-erbB-2 protein.

7. The sequence of claim 1, wherein the antibody is that produced by a hybridoma cell line bearing A.T.C.C. Accession No. HB10646.

8. The sequence of claim 1, wherein the sequence further encodes framework regions of an immunoglobulin.

9. The sequence of claim 8, wherein the framework regions are derived from a human immunoglobulin.

10. The sequence of claim 1, wherein the sequence further includes a control sequence for expression.

11. The sequence of claim 10, wherein the control sequence is a promoter.

12. The sequence of claim 10, wherein the control sequence is an enhancer.

13. A recombinant DNA sequence that encodes an antibody light chain variable region specific for c-erbB-2 protein.

14. The sequence of claim 13, wherein the light chain is specific for c-erbB-2 protein when combined with a heavy chain variable region, including that set forth in Sequence ID No. 3.

15. The recombinant DNA sequence of claim 13, that encodes an antibody light chain variable region which when incorporated into immunoglobulin conformation competes with an antibody produced by a hybridoma cell line bearing A.T.C.C. Accession No. HB10646 for the binding to c-erbB-2.

16. A recombinant DNA sequence that comprises DNA encoding an antibody light chain variable region to c-erbB-2 protein having an amino acid sequence consisting essentially of that sequence set forth in Sequence ID No. 4.

17. The recombinant DNA sequence of claim 16, wherein a coding strand sequence is that set forth in Sequence ID No. 2 at base positions 370 to 659.

18. The sequence of claim 13, wherein the sequence comprises a CDR region selected from the group consisting of those that encode the amino acid sequence at about position 24 to 34, 50 to 56, and 89 to 97 set forth on Sequence ID No. 4.

19. A recombinant DNA sequence that encodes an antibody heavy chain variable region specific for c-erbB-2 protein.

20. The sequence of claim 19, wherein the heavy chain is specific for c-erbB-2 protein when combined with a light chain variable region specific for c-erbB-2, set forth in Sequence ID No. 4.

21. The recombinant DNA sequence of claim 19, that encodes an antibody heavy chain variable region which when incorporated into immunoglobulin conformation competes with an antibody produced by a hybridoma cell line bearing A.T.C.C. Accession No. HB10646 for the binding to c-erbB-2.

22. A recombinant DNA sequence that comprises DNA encoding an antibody heavy chain variable region to c-erbB-2 having an amino acid sequence consisting essentially of that sequence set forth in Sequence ID No. 3.

23. The recombinant DNA sequence of claim 22 wherein a coding strand for the amino acid sequence is that set forth in Sequence ID No. 1 at about base positions 312 to 597.

24. The sequence of claim 19, wherein the sequence comprises a CDR region selected from the group consisting of those that encode the amino acid sequence at about position 31 to 35, 50 to 65, and 98 to 105 set forth on Sequence ID No. 3.

25. A recombinant DNA vector that comprises the DNA sequence of claim 1.

26. A recombinant DNA vector that comprises the DNA sequence of claim 13.

27. A recombinant DNA vector of claim 23 that further comprises a promoter or transcriptional activating sequence positioned to drive the expression of the DNA.

28. A recombinant DNA vector of claim 27 wherein one of the transcriptional activating sequences is an antibody enhancer.

29. A recombinant DNA vector that comprises the DNA sequence of claim 19.

30. A recombinant DNA vector that comprises the DNA sequence of claim 22.

31. A recombinant DNA vector of claim 29 that further comprises a promoter and transcriptional activating sequence positioned to drive the expression of said DNA.

32. A rec mbinant DNA vector of claim 31, wherein one of the transcriptional activating sequences is an antibody enhancer.

33. A recombinant DNA sequence that comprises DNA encoding a chimeric c-erbB-2 specific heavy chain peptide, the variable region derived from a first genetic source and a constant region derived from a second and different genetic source.

34. The recombinant DNA sequence of claim 33, wherein the heavy chain variable region has an amino acid sequence consisting essentially of Sequence ID No. 3.

35. The recombinant DNA sequence of claim 34, wherein a coding strand for the amino acid sequence is that of Sequence ID No. 1 at about base positions 312 to 597.

36. The recombinant DNA sequence of claim 33, wherein the heavy chain variable region has a CDR region selected from the group consisting of those that encode for the amino acid sequence at about position 31 to 35, 50 to 65, and 98 to 105 set forth on Sequence ID No. 3.

37. A recombinant DNA vector that comprises the DNA sequence of claim 33.

38. A recombinant DNA sequence that comprises DNA encoding a chimeric c-erbB-2 specific light chain peptide, a variable region derived from a first genetic source and a constant region derived from second and different genetic source.

39. The recombinant DNA sequence of claim 38, wherein the light chain variable region has an amino acid sequence consisting essentially of Sequence ID No. 4.

40. The recombinant DNA sequence of claim 39, wherein a coding strand for the amino acid sequence is that of Sequence ID No. 2 at about base positions 370 to 659.

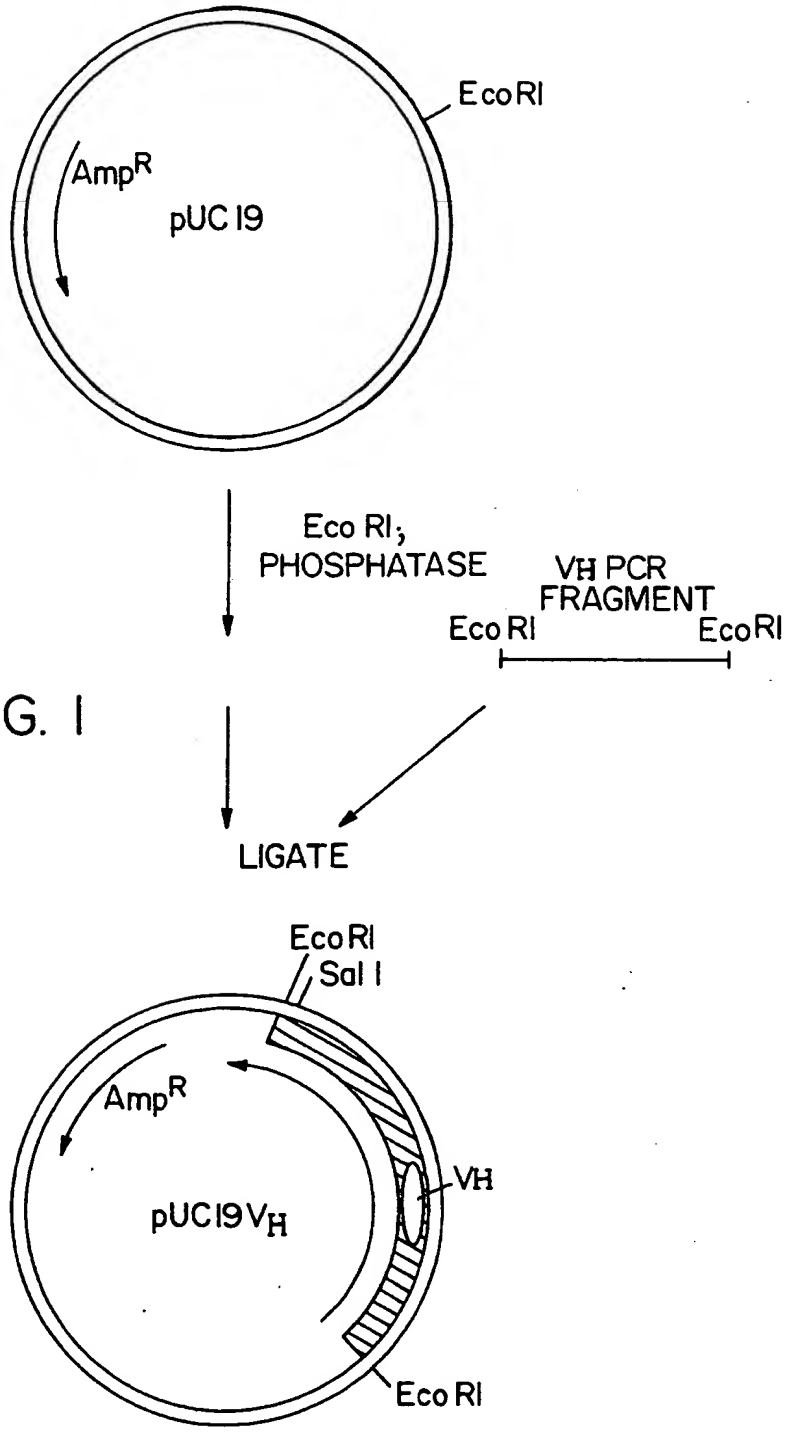
41. The recombinant DNA sequence of claim 38, wherein the light chain variable region has a CDR region selected from the group consisting of those that encode for the amino acid sequence at about position 24 to 34, 50 to 56, and 89 to 97 set forth on Sequence ID No. 4.

42. A recombinant DNA vector that comprises the DNA sequence of claim 38.

43. A host cell that expresses chimeric antibodies chains specific for c-erbB-2.

44. The host cell line of claim 43, that expresses antibody which when in immunoglobulin conformation competes with an antibody produced by the hybridoma cell line bearing A.T.C.C. Accession No. HB10646 for the binding to c-erbB-2.

45. A host cell that expresses the protein encoded by the sequence of claim 1.


EcoRI
AmpR
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PHOSPHATASE
VH PCR
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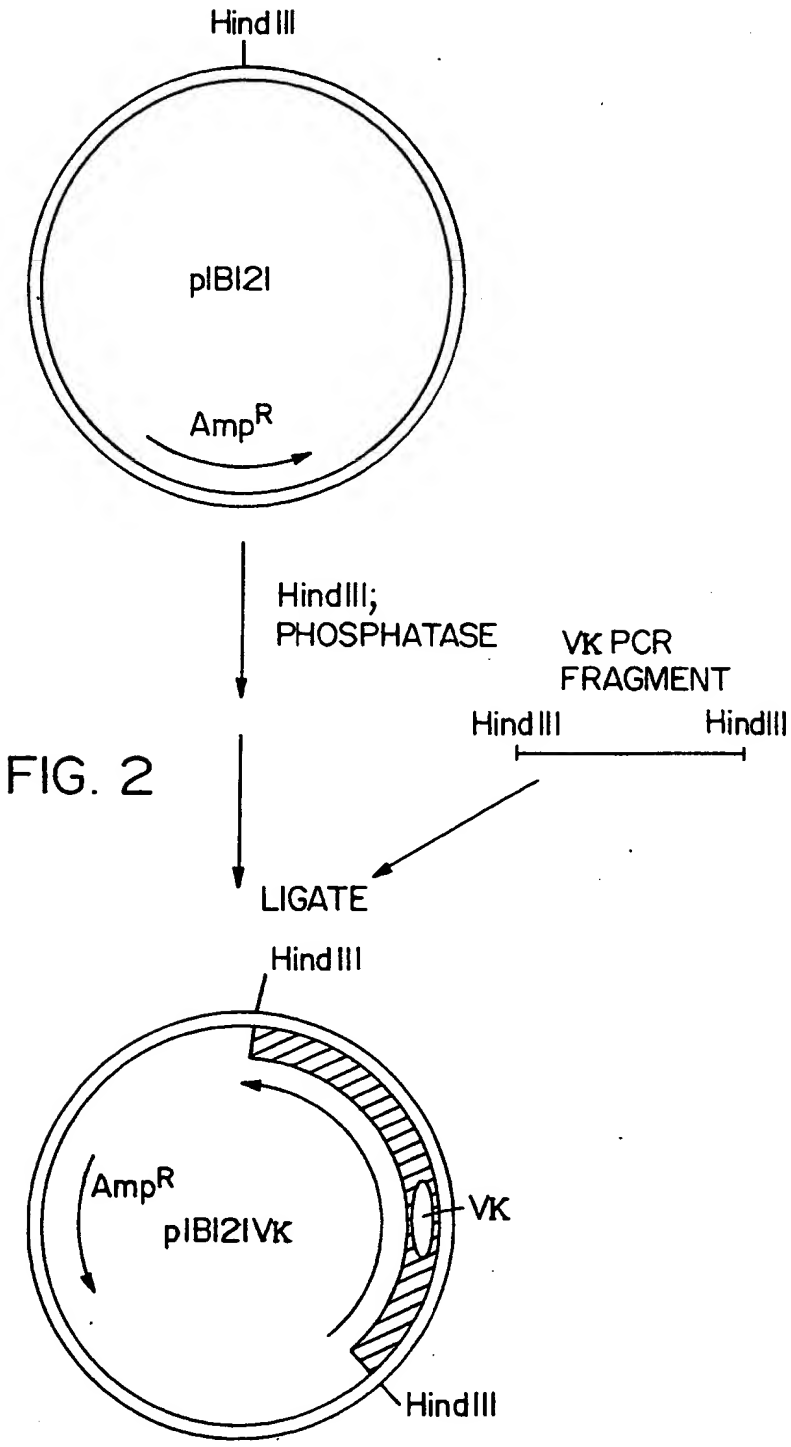
Eco RI

FIG. 1


Hind III
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Amp^R
Hind III;
PHOSPHATASE
VK PCR
FRAGMENT
Hind III
Hind III
LIGATE
Hind III
Amp^R
pIBI21VK
VK
Hind III

FIG. 2

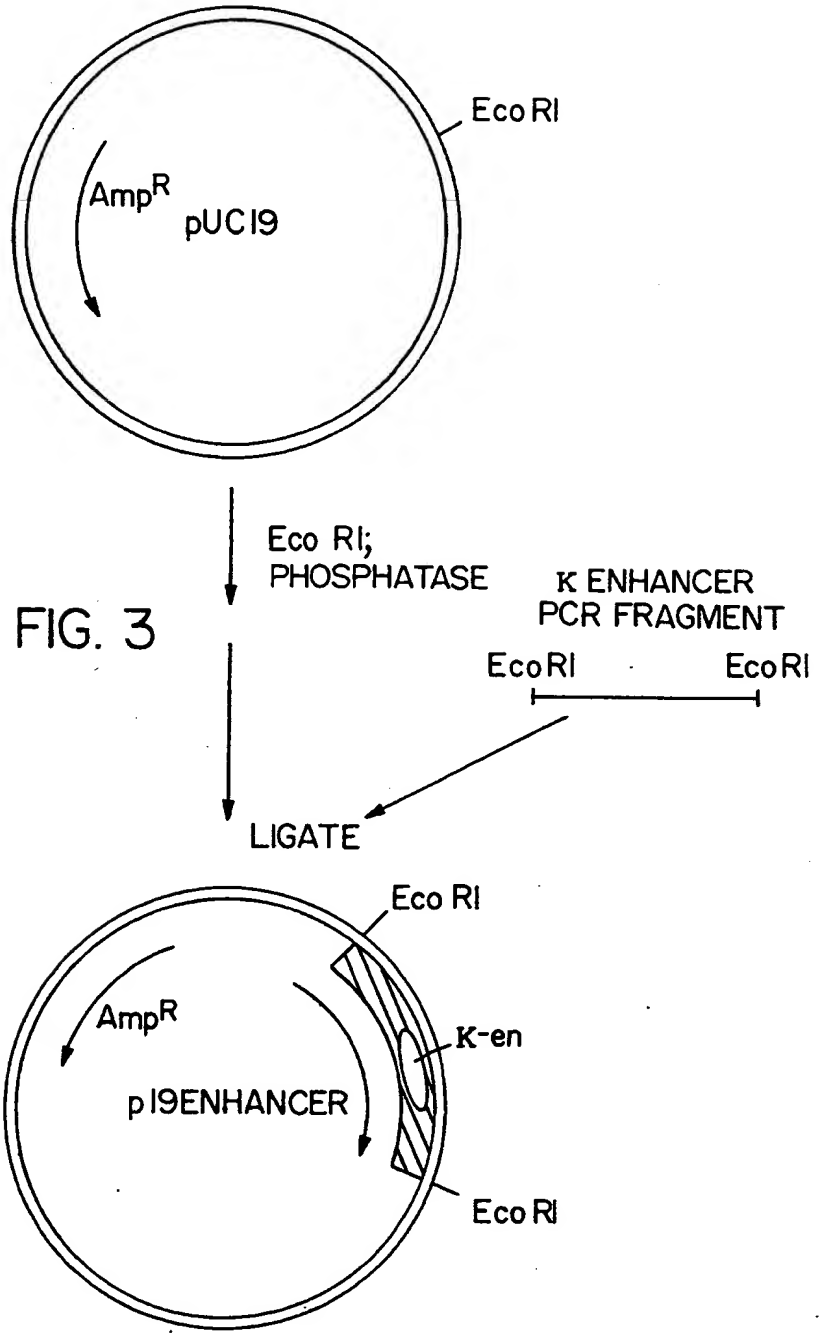
Eco RI
Amp^R
pUC19
Eco RI;
PHOSPHATASE
κ ENHANCER
PCR FRAGMENT
Eco RI
Eco RI
LIGATE
Eco RI
Amp^R
κ-en
p19ENHANCER
Eco RI

FIG. 3

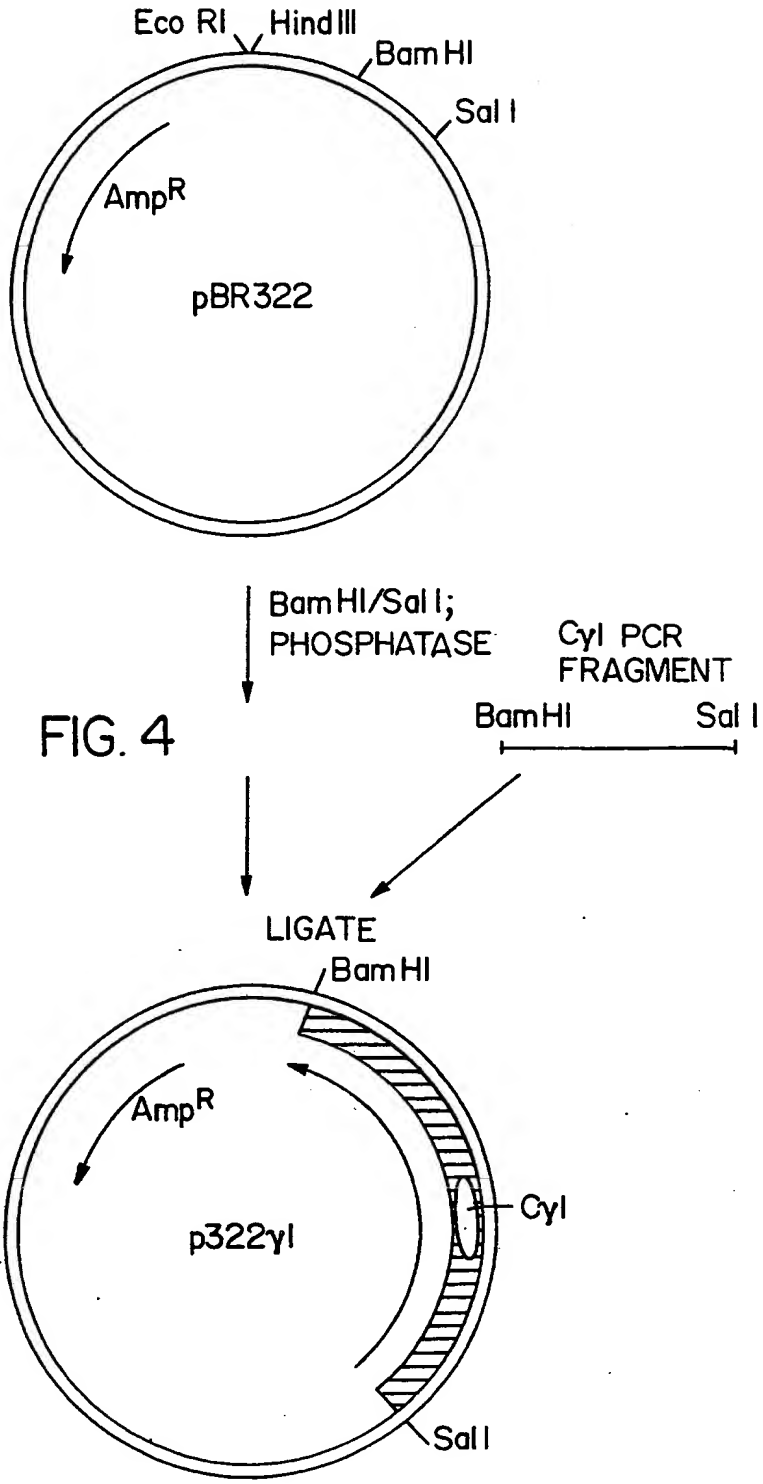
Eco RI
Hind III
Bam HI
Sal I
Amp^R
pBR322
Bam HI/Sal I;
PHOSPHATASE
Cyl PCR
FRAGMENT
Bam HI
Sal I
LIGATE
Bam HI
Amp^R
Cyl
p322yl
Sal I

FIG. 4

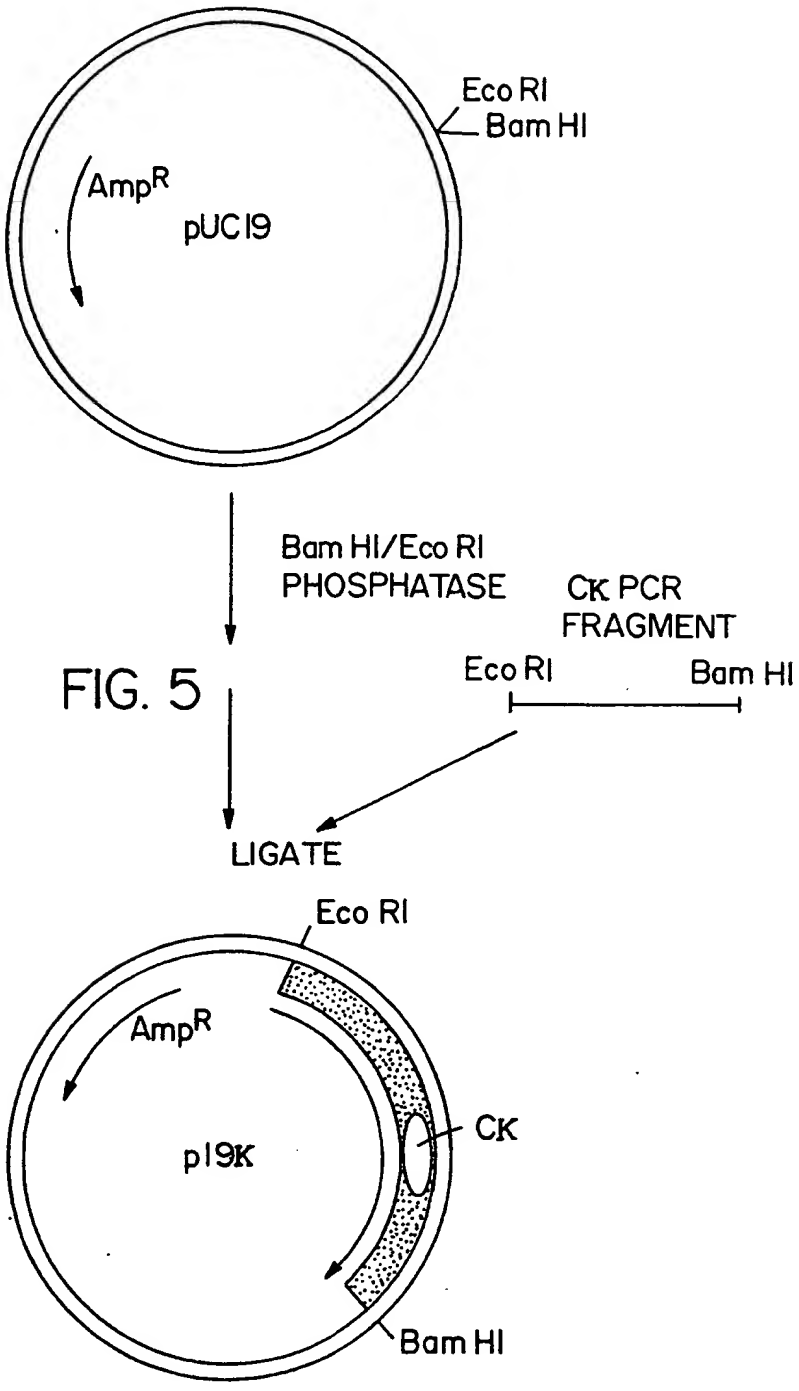
Eco RI
Bam HI
AmpR
pUC19
Bam HI/Eco RI
PHOSPHATASE
CK PCR
FRAGMENT
Eco RI
Bam HI
LIGATE
Eco RI
AmpR
CK
p19K
Bam HI

FIG. 5

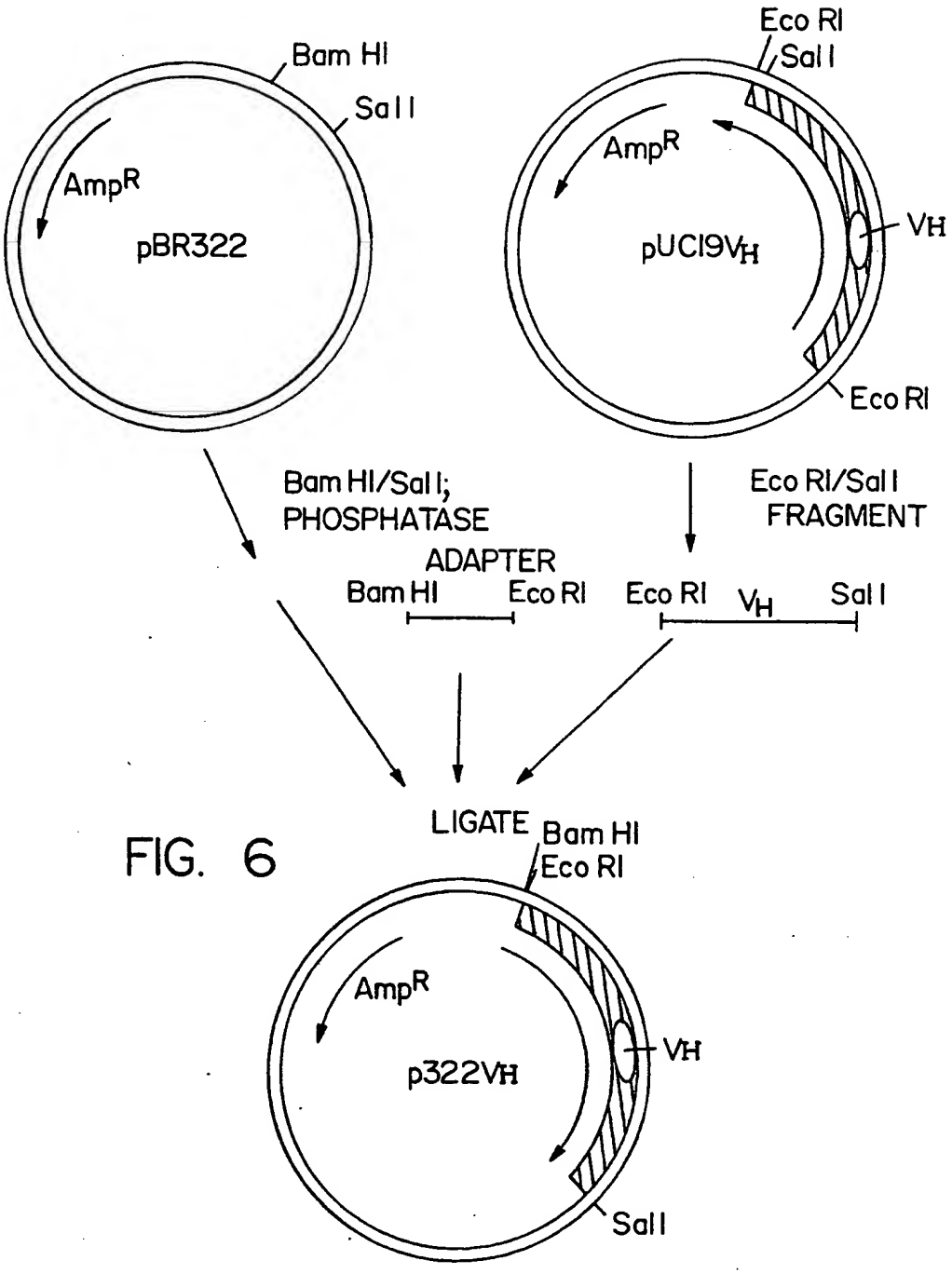
Bam HI
Sal I
AmpR
pBR322
Eco RI
Sal I
AmpR
pUC19VH
VH
Eco RI
Bam HI/Sal I;
PHOSPHATASE
Eco RI/Sal I
FRAGMENT
ADAPTER
Bam HI
Eco RI
Eco RI
VH
Sal I
LIGATE
Bam HI
Eco RI
AmpR
p322VH
VH
Sal I

FIG. 6

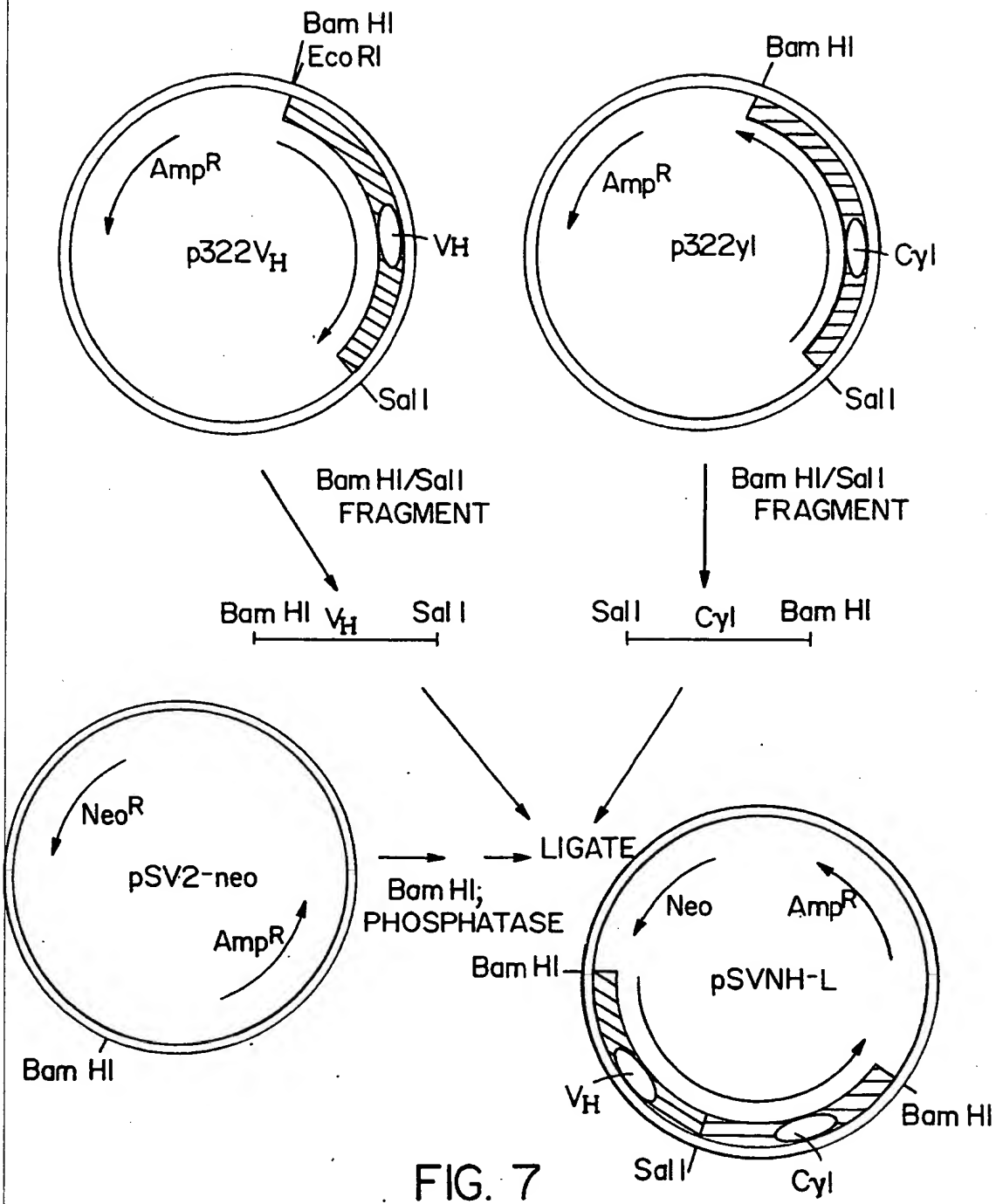
Bam HI
Eco RI
Amp^R
p322V_H
VH
Sal I
Bam HI
Amp^R
p322γ1
Cγ1
Sal I
Bam HI/Sal I FRAGMENT
Bam HI/Sal I FRAGMENT
Bam HI V_H Sal I
Sal I Cγ1 Bam HI
Neo^R
pSV2-neo
Amp^R
Bam HI
Bam HI; PHOSPHATASE
LIGATE
Neo
Amp^R
Bam HI
pSVNH-L
V_H
Bam HI
Sal I
Cγ1

FIG. 7

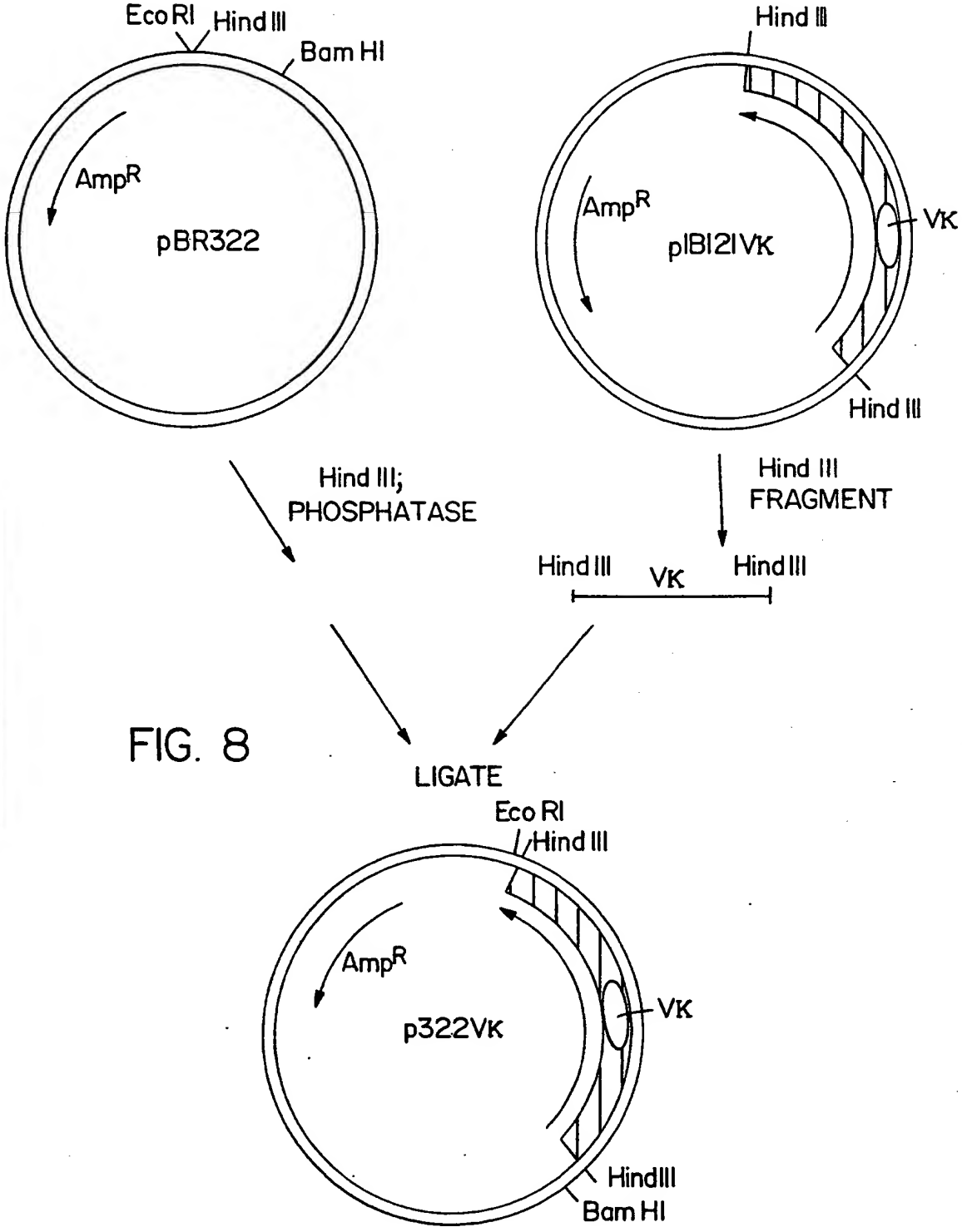
Eco RI
Hind III
Bam HI
AmpR
pBR322
Hind III
AmpR
pIBI21VK
VK
Hind III
Hind III;
PHOSPHATASE
Hind III
FRAGMENT
Hind III
VK
Hind III
LIGATE
Eco RI
Hind III
AmpR
p322VK
VK
HindIII
Bam HI

FIG. 8

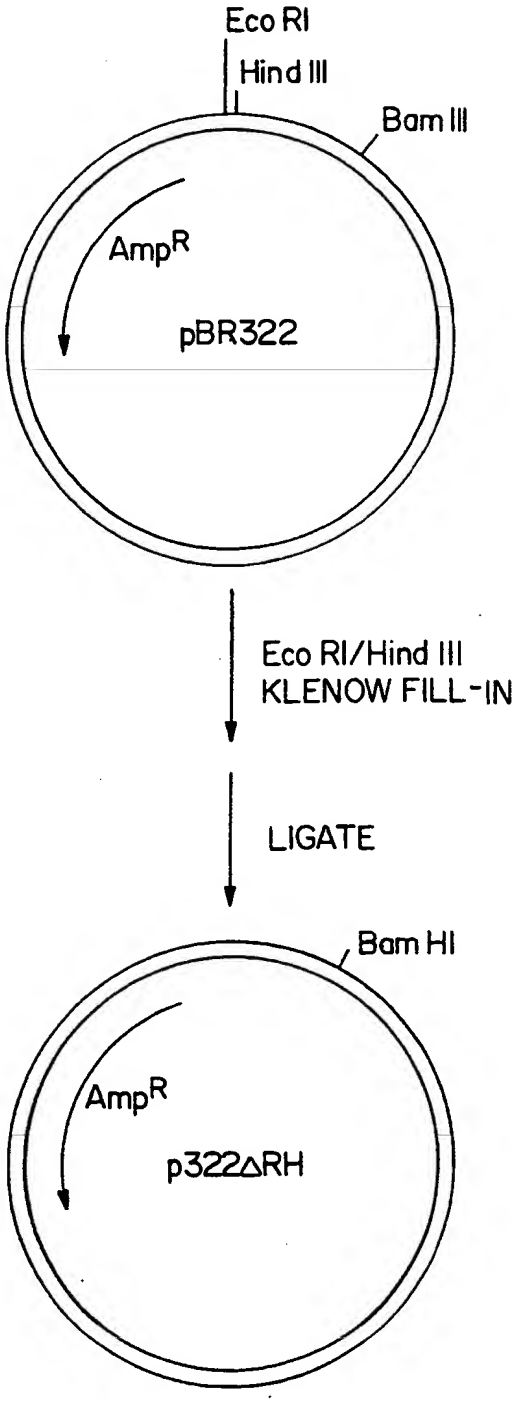
Eco RI
Hind III
Bam III
AmpR
pBR322
Eco RI/Hind III
KLENOW FILL-IN
LIGATE
Bam HI
AmpR
p322ΔRH

FIG. 9

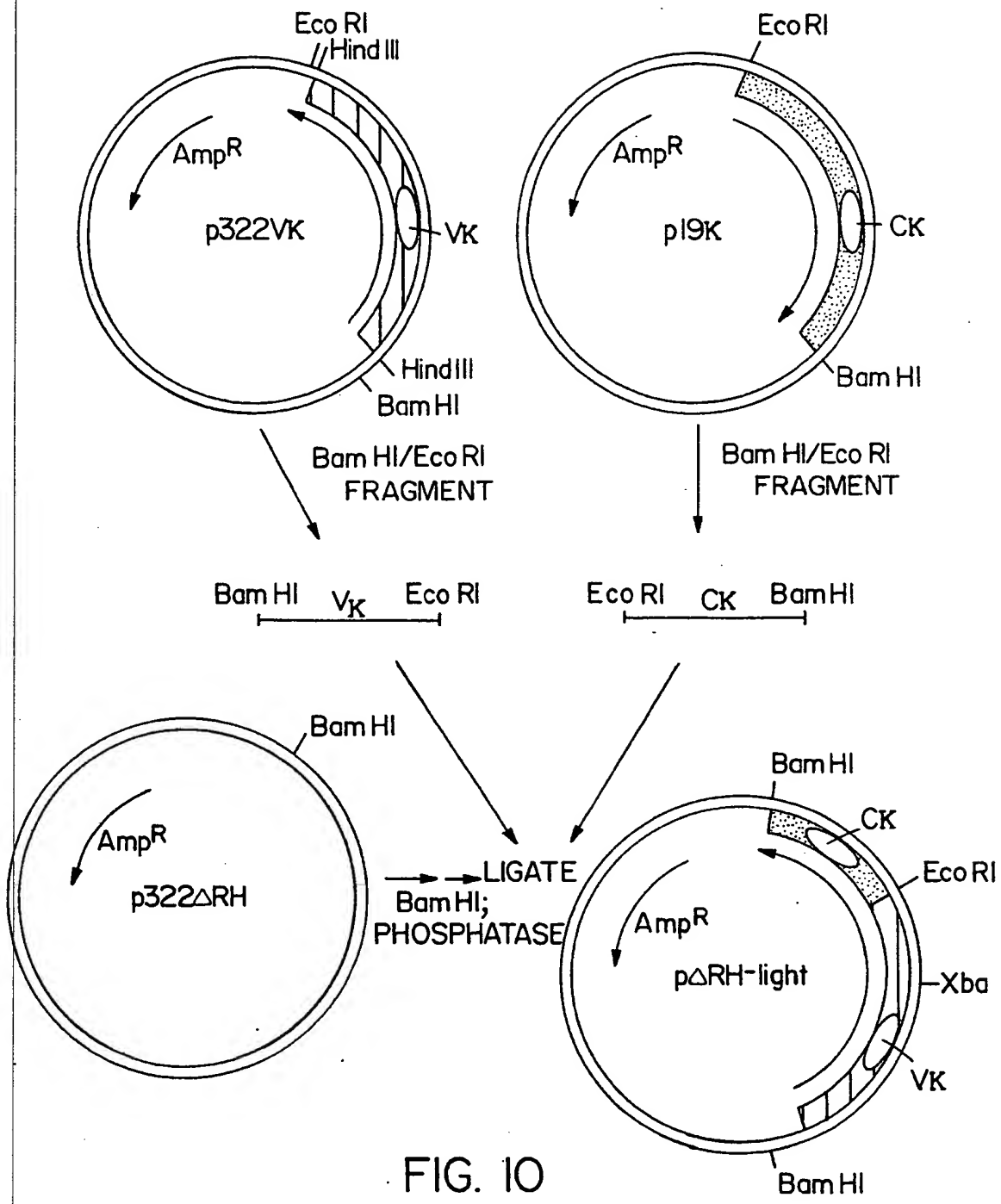
Eco RI
Hind III
AmpR
p322VK
VK
Hind III
Bam HI
Bam HI/Eco RI
FRAGMENT
Eco RI
AmpR
p19K
CK
Bam HI
Bam HI/Eco RI
FRAGMENT
Bam HI VK Eco RI
Eco RI CK Bam HI
Bam HI
AmpR
p322ΔRH
LIGATE
Bam HI;
PHOSPHATASE
Bam HI
CK
Eco RI
AmpR
pΔRH-light
Xba
VK
Bam HI

FIG. 10

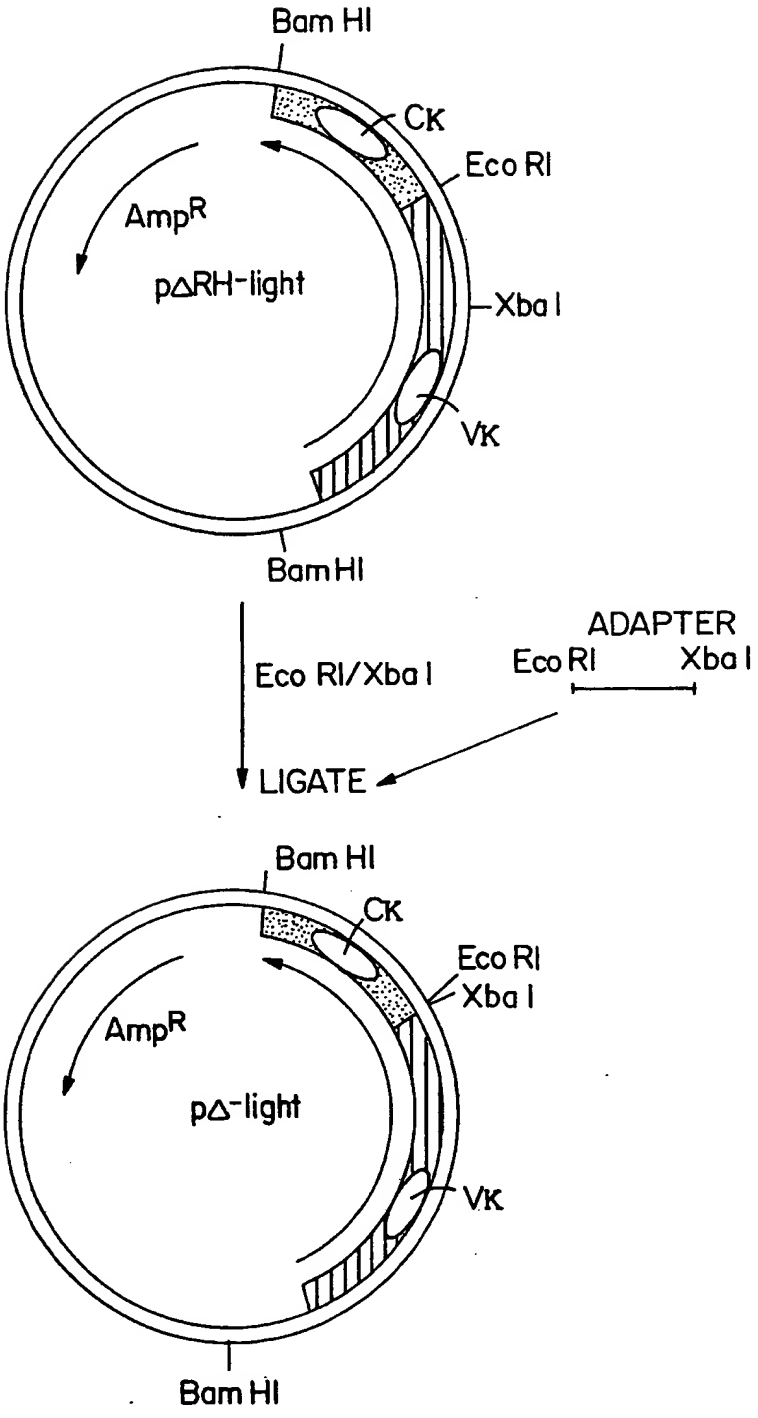
Bam HI
CK
Eco RI
AmpR
pΔRH-light
Xba I
VK
Bam HI
ADAPTER
Eco RI
Xba I
Eco RI/Xba I
LIGATE
Bam HI
CK
Eco RI
Xba I
AmpR
pΔ-light
VK
Bam HI

FIG. 11

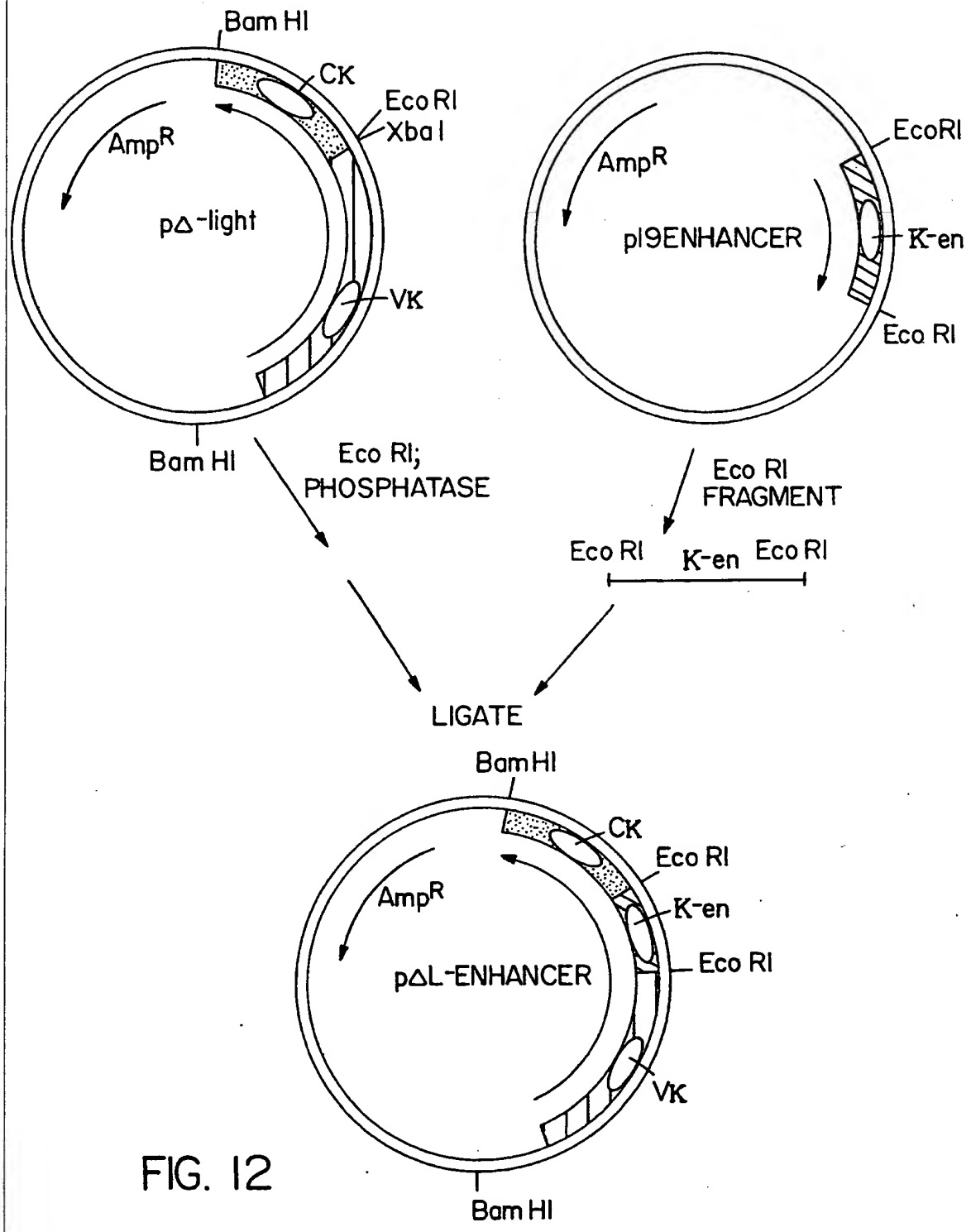
Bam HI
CK
Eco RI
Xba I
AmpR
pΔ-light
VK
Bam HI
Eco RI;
PHOSPHATASE
EcoRI
AmpR
pI9ENHANCER
K-en
Eco RI
Eco RI
FRAGMENT
Eco RI
K-en
Eco RI
LIGATE
BamHI
CK
Eco RI
AmpR
K-en
pΔL-ENHANCER
Eco RI
VK
Bam HI

FIG. 12

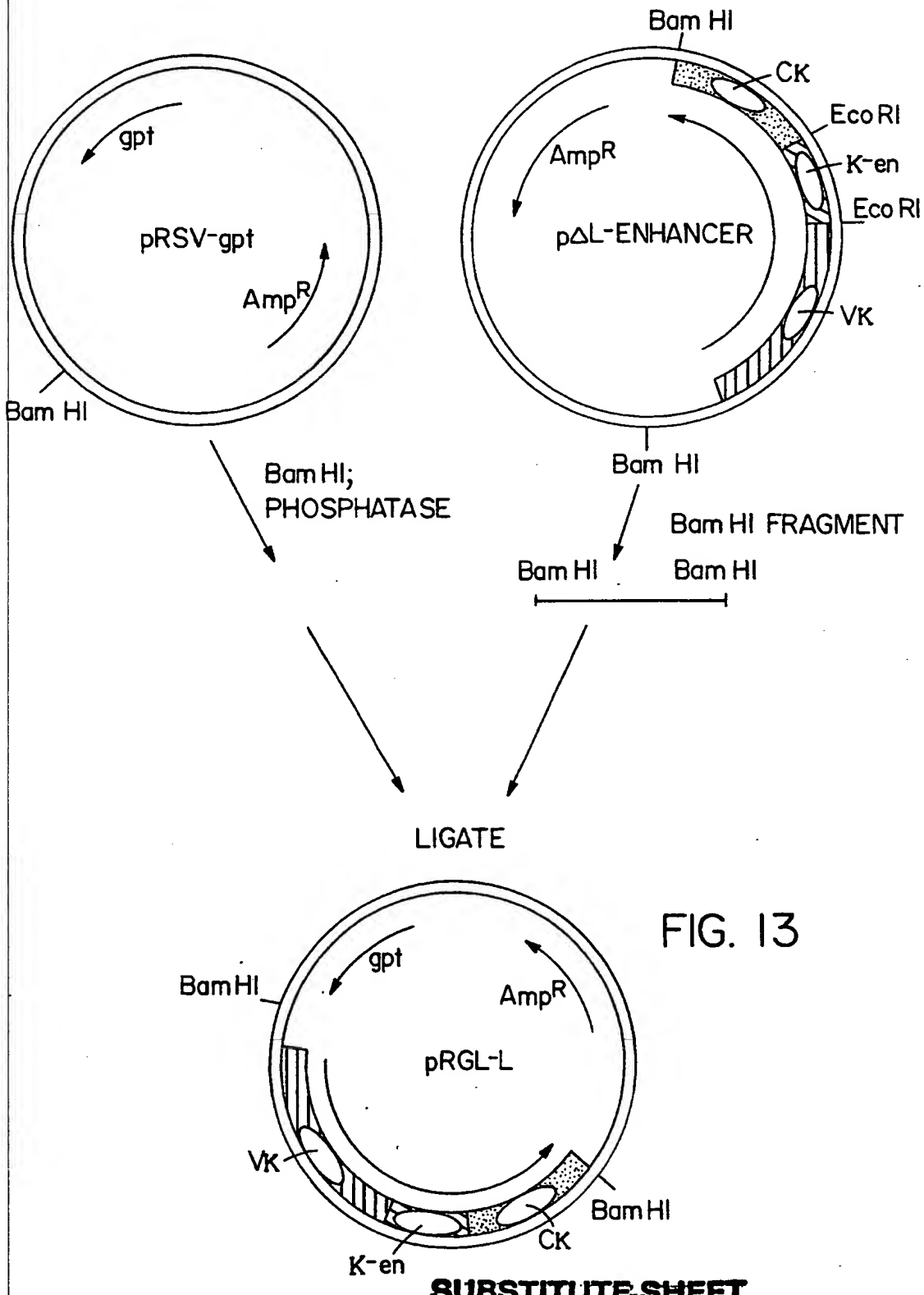
gpt
pRSV-gpt
AmpR
Bam HI
Bam HI
CK
Eco RI
K-en
Eco RI
AmpR
pΔL-ENHANCER
VK
Bam HI
Bam HI; PHOSPHATASE
Bam HI FRAGMENT
Bam HI
Bam HI
LIGATE
gpt
Bam HI
AmpR
pRGL-L
VK
Bam HI
CK
K-en

FIG. 13

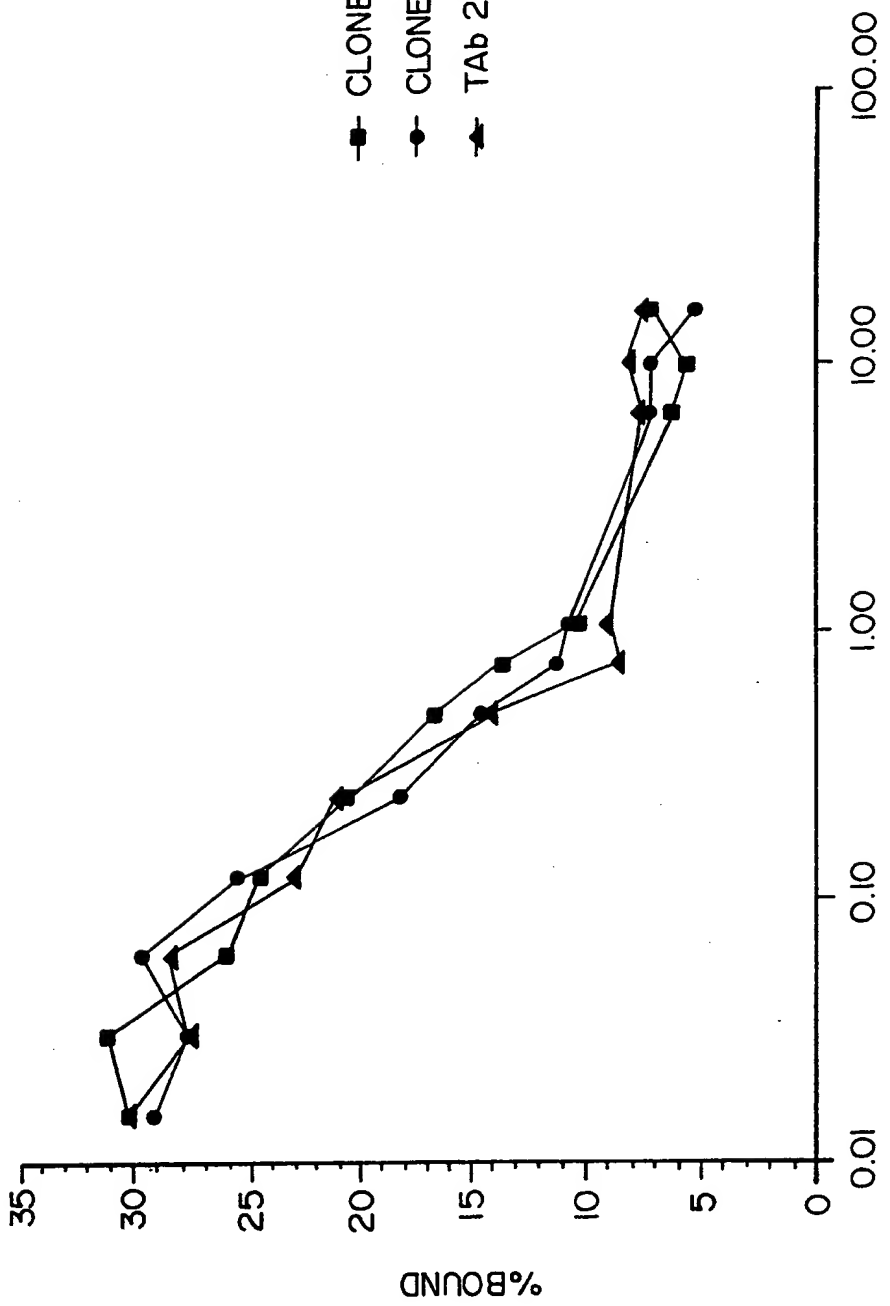
CLONE E8
CLONE A7
TAb 250
%BOUND
0
5
10
15
20
25
30
35
0.01
0.10
1.00
10.00
100.00
COLD ANTIBODY (µg/ml)

FIG. 14

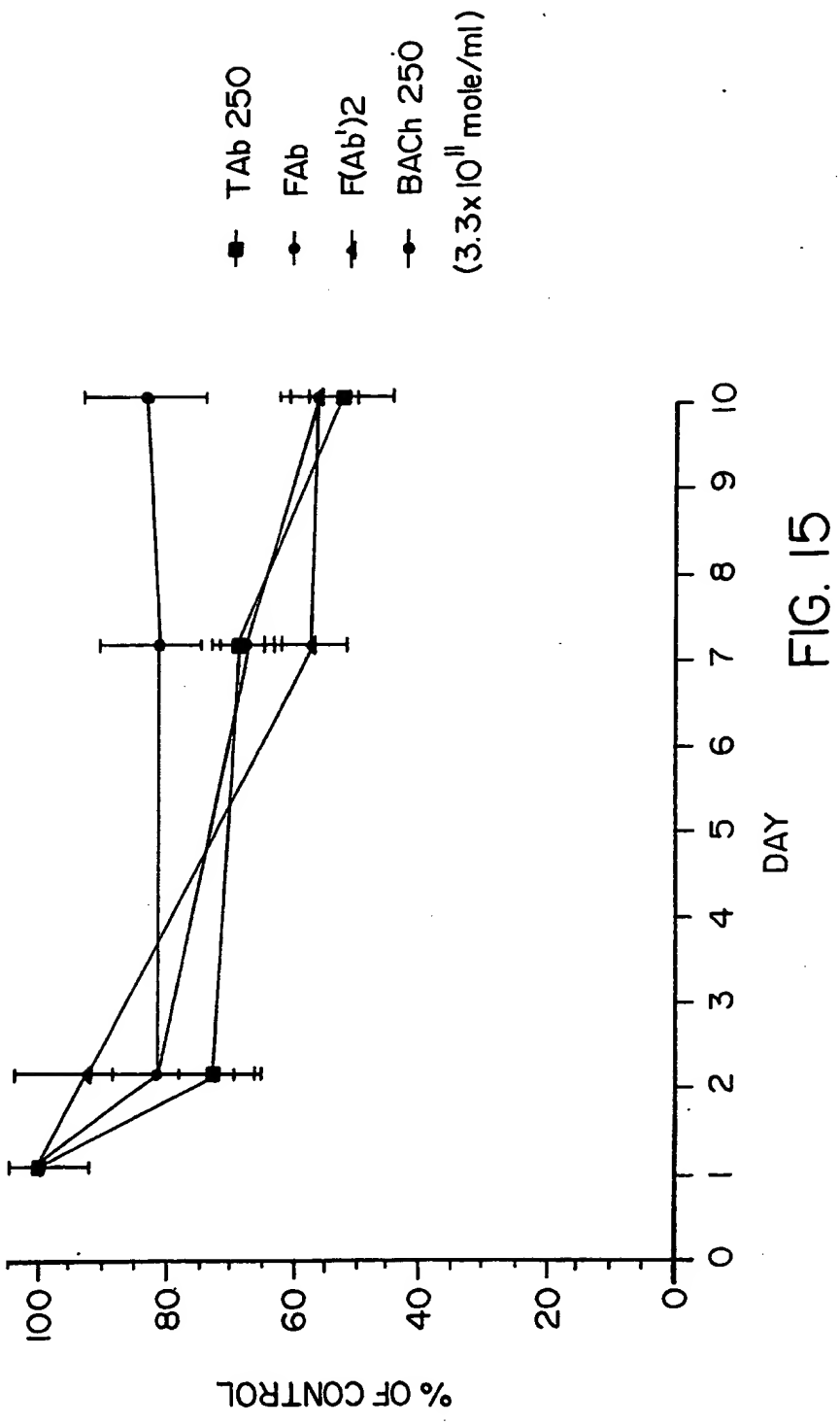
TAb 250
FAb
F(Ab')2
BACh 250
(3.3x10^11 mole/ml)
% OF CONTROL
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
DAY

FIG. 15

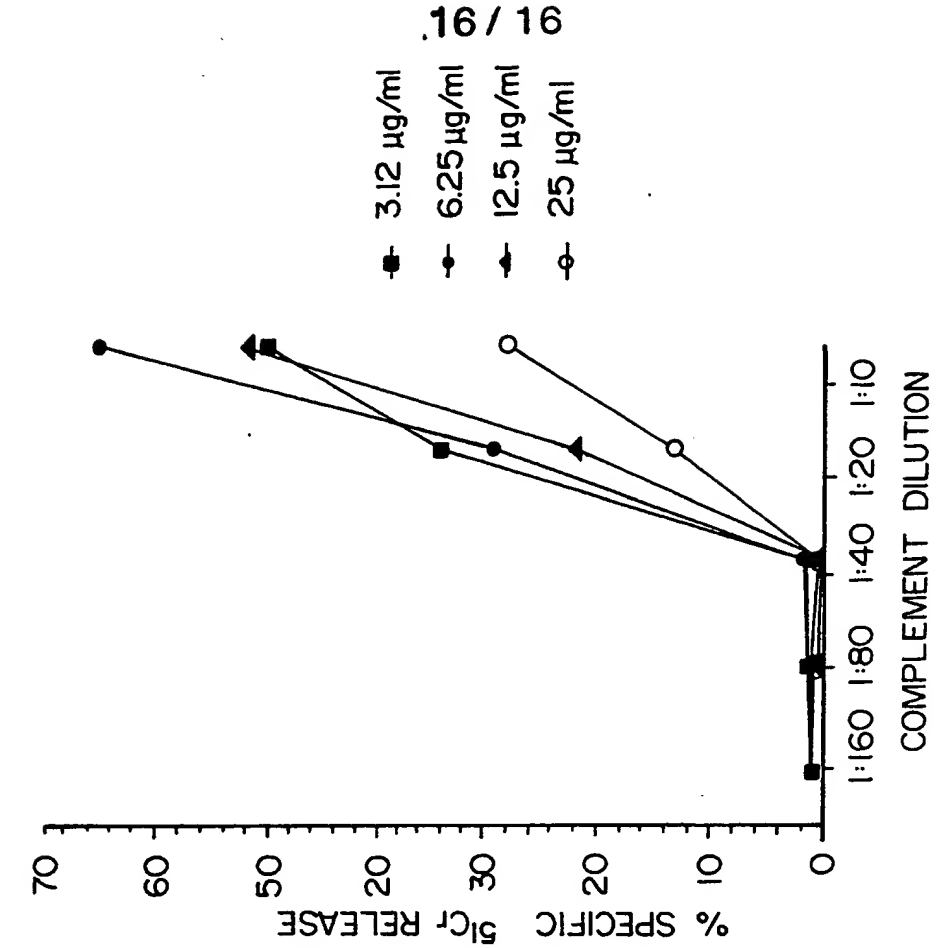

FIG. 16B

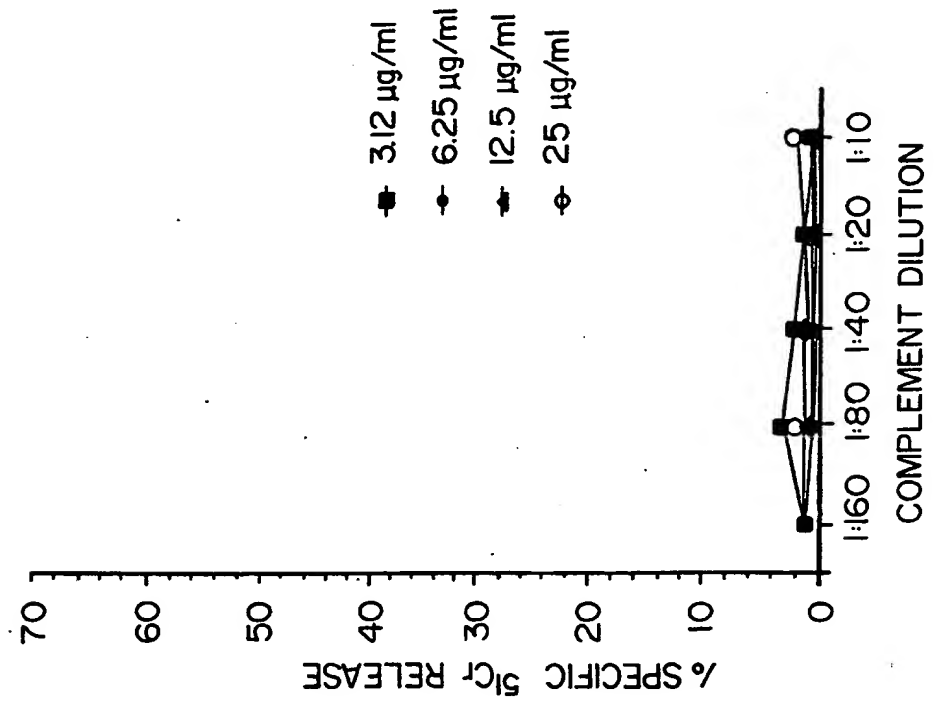

FIG. 16A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10437

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 5/12; C12P 21/02
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.6, 70.21, 172.2, 240.27; 536/27, 530/387.3, 388.22, 389.7, 389.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and Medline
search terms: c-erb-2, chimeric, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Volume 4, issued 1989, S. J. McKenzie et al. "Generation and characterization of monoclonal antibodies specific for the human neu oncogene product, p185", pages 543-548, see entire document	1-45
Y	Oncogene, issued 1990, F. van Leeuwen et al. "Mutation of the human new protein facilitates down-modulation by monoclonal antibodies", pages 497-503, see entire document.	1-45
Y	Science, Volume 230, issued 06 December 1985, L. Coussens et al. "Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene", pages 1132-1139, see entire document.	1-45
Y	WO, A, 91/09967 (Adair et al.) 11 July 1991, see entire document	1-45
Y	Harlow et al. "Antibodies: A laboratory Manual", published 1988 by Cold Spring Harbor Laboratory (N.Y.), see pages 72-77, 92-97, 128-135 and 141-157.	1-45

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1993	02 FEB 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DONALD E. ADAMS, PH.D.
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10437

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.6, 70.21, 172.2, 240.27; 536/27, 530/387.3, 388.22, 389.7, 389.1